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A Nutrition Survey in East York Township

1. Description of Survey and General Statement of Results

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DURING the past ten years there has been an increasing interest in the public health aspects of nutrition; one phase of this interest has been an attempt to ascertain the extent and kinds of malnutrition existing in countries with a fairly high standard of living and with comparatively little acute under-nutrition. The important report of Orr (1) on dietary conditions in Great Britain paved the way for extensive examinations of food habits in various countries. The large-scale surveys of Stiebeling (2) in the United States followed a similar pattern, as did five Canadian studies (3) made in the past five years. All of these were concerned with food consumption and comparisons were made with currently accepted nutritional standards to determine the adequacy of food intakes. From such data conclusions have been drawn regarding dietary deficiencies; for example, statements have been made that Canadian low-income families, at least in urban communities, have deficiencies of calcium, iron, and of most of the vitamins. This type of inference has been criticized because the information was derived only from an examination of food consumption; the state of nutrition of an individual may be greatly different from that shown from the diet. With this distinction in mind, a number of procedures have been proposed for detecting malnutrition other than by inspection of the diet. These have been tested separately and combinations of them have been employed in several surveys in the United States, more particularly by Kruse (4), by Milam (5), and by Youmans (6). A nutritional survey of this general type, including dietary studies, physical, medical and dental examinations, and special

diagnostic tests, had not previously been attempted in Canada. This study was undertaken for two principal purposes: to test the validity of several recommended procedures and to determine whether the results of dietary studies would be in agreement with those obtained by other methods of nutritional appraisal.

After the establishment of a health unit in East York Township with the co-operation of the Ontario Department of Health, the Rockfeller Foundation, and the School of Hygiene of the University of Toronto, an opportunity was presented to conduct a nutritional study. A recent dietary survey in Toronto had indicated that adolescents secured less adequate food supplies than younger children or adults. It was accordingly decided to operate in the adolescent age group. With the kind permission of the East York Board of Education, the study was carried out in the East York Collegiate Institute. Appreciation is expressed to the Board of Education and to the principal and teachers of the school for the splendid co-operation which was continuously evident. The school nurse, Miss Sara Oliphant, was of great assistance throughout the study and sincere thanks are expressed to her.

The population of East York Township, an urban suburb immediately east of Toronto, is largely middle-class and Anglo-Saxon. The Collegiate Institute has an enrolment of approximately 1400 students; the building is modern, well-equipped and contains a cafeteria in which low-cost lunches are available. It was essential to secure permission of parents before the study was begun; this was done by asking each student to take home an authorization card which could be signed and returned. Permission was thus obtained for the examination of 850 students; of these, 546, chosen to give a representative sample with regard to age and sex, were completely studied (260 boys and 286 girls). The age distribution of these students is given in Table I.

TABLE I

AGE DISTRIBUTION OF STUDENTS EXAMINED IN SURVEY

Figures shown are percentages of students of each sex.

Age	*Girls*	*Boys*
11	0	0.4
12	4.2	1.2
13	16.1	11.5
14	21.7	28.8
15	21.7	29.6
16	22.4	15.0
17	9.1	10.8
18	5.3	2.3
19	0.4	0.4

DESCRIPTION OF PROCEDURES

The study comprised: (1) procurement of dietary records, (2) detailed physical examination with the securing of a medical history, (3) a dental examination, (4) special tests. Measurement of haemoglobin and routine urine examinations were made.

(1) *Dietary Records.* While it might have been desirable to secure weights

of food actually consumed, this was considered to be impracticable. Accordingly, the students were asked to record, in small booklets which were provided, all foods used during one week with a description of the sizes of servings. Emphasis was placed on inclusion of foods taken between meals and on sugar, cream or milk used in beverages. A trained and experienced nutritionist was present in the school throughout the period of collection of records to answer questions and to provide assistance.

(2) *Physical Examination.* An attempt was made to have this thorough and much more complete than a routine school examination. All of the points listed for physical examination by the Food and Nutrition Board of the U.S. National Research Council (7) were included. Examinations of girls were made by women physicians, of boys by men physicians.

(3) *Medical History.* This, again, was planned to be reasonably complete. Several special questions were included; for example, the number of colds, and an estimate of the amount of sleep generally secured.

(4) *Special Tests.* A slit-lamp examination of the eyes of all subjects was made to determine (a) occurrence of conjunctival lesions, described by Kruse (8) as indicative of vitamin A deficiency; (b) corneal vascularization, possibly due to riboflavin insufficiency. Measurements of capillary fragility were made by the suction-cup procedure. Dark adaptation was tested using the Wald-Stevens portable adaptometer.

(5) *Dental examinations* were made by dentists specially chosen through a co-operative arrangement with the Ontario Dental Association. Special attention was paid to abnormalities of the gums. Thanks are expressed to Dr. A. E. Higgins for his assistance in arranging for this examination.

In addition to the above, average academic standings for the current year were obtained through the courtesy of the principal of the Collegiate Institute.

The results of the study of each student were transmitted from the School of Hygiene to the Medical Officer of Health who, in turn, gave a copy of the individual report to the family physician, at the same time notifying the parents that this had been done. Where no physician was known, a specially worded report was sent directly to the parents.

Survey Results

(1) *Food Consumption*

The records of food intake were transferred to special forms for the calculation of nutritive values. Figures for food composition in terms of calories, protein, fat, carbohydrate, calcium, phosphorus and iron were taken from the Tables of Food Value of the Canadian Council of Nutrition, those for vitamins from two tables published by Munsell (9); all values used were for raw foods. While the absolute values of food consumption are of interest, it is essential to assess them in terms of adequacy. This was done by comparison with the recommended daily allowances of the U.S. National Research Council, as approved for use in Canada by the Council on Nutrition. All values have been expressed as percentages of the recommended daily allowances and classified

as follows: excellent, 90 per cent or more of the standard; good, 80-90 per cent; fair, 70-80 per cent; poor, below 70 per cent. An attempt has been made to evaluate the composite value of the food consumption by the use of a "diet score." This was the average percentage of adequacy for calories, protein, calcium, iron, vitamin A, thiamin, riboflavin, and ascorbic acid. In calculating the average any individual value above 100 was taken as 100; this was done to prevent undue weighting by an excess of any one constituent. The use of this "diet score" can, of course, be criticized on several grounds. It is not based on a complete picture of the food consumption, it gives equal weighting to the various dietary values used, and it may obscure a marked deficiency in one constituent. It is, however, a debatable question as to how a record of food consumption can be evaluated to give a composite rating and the method which was used is probably as fair as any other current procedure. Its use has, however, been made with considerable reservation and undue reliance has not been placed upon it.

Data accruing from the evaluation of the food consumption are given in Table II. In both girls and boys the most striking findings were the low consumption of foods containing thiamin and ascorbic acid. Intakes of protein, iron and vitamin A were reasonably high, although the supplies of iron secured by girls were less than in the case of boys. Amounts of calcium and of riboflavin were fair. Fifteen per cent of all the students examined had extra supplies of vitamin A and D in the form of cod liver oil or a fish liver preparation.

TABLE II

GRADING OF NUTRITIVE VALUE OF FOOD CONSUMPTION

A. GIRLS

	Excellent	*Good*	*Fair*	*Poor*
Calories	55.4 p.c.	25.2 p.c.	11.5 p.c.	8.1 p.c.
Protein	71.0	16.1	8.7	4.2
Calcium	52.8	12.6	9.8	24.9
Iron	47.9	24.9	15.7	11.5
Vitamin A	86.8	4.2	3.5	8.7
Thiamin	12.6	12.2	19.2	56.0
Riboflavin	64.0	8.7	10.5	16.8
Ascorbic Acid	8.7	5.9	10.5	74.8
Diet Score	16.1	55.4	22.1	6.6

B. BOYS

	Excellent	*Good*	*Fair*	*Poor*
Calories	49.7 p.c.	21.9 p.c.	16.1 p.c.	12.3 p.c.
Protein	81.2	8.1	8.5	2.3
Calcium	49.5	16.3	12.3	22.7
Iron	82.7	7.7	8.1	1.5
Vitamin A	84.7	7.7	4.6	3.1
Thiamin	8.5	6.2	15.0	70.4
Riboflavin	42.7	20.4	13.1	23.8
Ascorbic Acid	7.7	6.5	5.4	80.4
Diet Score	11.2	60.5	25.9	3.1

Calculations of the consumption of several common foods were made and expressed in average daily amounts per person. In the case of bread the

average consumption was: for boys, 5 slices of white and 1 1/5 slices of brown bread; for girls, 3¾ slices of white and 4/5 slice of brown bread. The use of milk was reasonably generous; calculated to include all milk used as a beverage, but not in cooking, the average daily amount for girls was 0.9 pints and for boys 1 pint.

It seemed useful to ascertain whether the use of poor diets was greater among older students; this was not found to be the case, since the distribution of various grades of diets was approximately the same in each age group. The same general statement was found to be true for calcium intakes, indicating that the use of milk was not less in the older age groups.

(2) *Physical Examination and Medical History*

While a thorough physical examination and a complete medical history were obtained, only those findings which were considered to be related to nutritional status will be reported.

(a) *Height and Weight.* For evaluation of height and weight the Baldwin-Wood Tables as cited by Roberts (10) were employed. A leeway of plus or minus 10 per cent was allowed in deciding that an individual varied from "normal" weight. In grading height the leeway shown in the Baldwin-Wood Tables was used. As assessed on these bases each student was graded, for both height and weight, as normal, above normal, or below normal. Results are given in Table III. With respect to height, 95 per cent of all the students were normal or above normal, and about one-fourth of the group were assessed as underweight.

TABLE III

Grading of Weight and Height of Examined Students

Figures shown are percentages of students for each sex.

	Height		*Weight*	
Grading	*Girls*	*Boys*	*Girls*	*Boys*
Above normal	21.0	15.8	5.9	8.5
Normal	72.7	78.5	62.9	70.0
Below normal	5.6	5.7	30.4	21.5

(b) *Nutritional Status as Judged by Physical Examination.* Each examining physician was requested to grade each student as excellent, good, fair or poor with regard to nutritional condition. The results of this grading are given in Table IV. It will be noted that four-fifths of the group were considered to be in excellent or good nutritional status.

TABLE IV

Appraisal of Health and Nutritional Status by Physical Examination

		Girls	*Boys*
Health:	Excellent	14 p.c.	37 p.c.
	Good	64	48
	Fair	21	14
	Poor	1	0
Nutritional Status:	Excellent	52 p.c.	65 p.c.
	Good	26	12
	Fair	20	22
	Poor	2	1

(c) *General State of Health as Judged by Physical Examination.* The general health of the students was graded as excellent, good, fair or poor. The results are given in Table IV. In this case, also, four-fifths of the group were stated to be in excellent or good health. None of the boys and only 1 per cent of the girls were considered to have "poor" health.

(d) *Haemoglobin.* To avoid difficulties of transportation, and for convenience, all haemoglobin measurements were made with a Dare haemoglobinometer. It was realized that accurate results could not be obtained with this instrument but it was believed that the determinations were sufficiently reliable for the purpose intended. Seventy-five per cent of the girls and 97 per cent of the boys had haemoglobins over 90 per cent. There were no values below 70 per cent.

(e) *Physical Defects.* Eleven per cent of the girls and 12 per cent of the boys showed physical defects, but most of these were of a very minor nature, such as weak arches.

(3) *Dental Examination*

No cases of marked gingivitis were seen. The situation with regard to cavities was much less satisfactory, since 50 per cent of all girls and boys examined had more than four active caries. Thirty-six per cent of the girls and 24 per cent of the boys had more than six filled cavities. Obviously, processes of dental decay were markedly evident and there was apparent a clear need for dental care.

(4) *Special Tests*

The results of these will be considered in the following section.

CORRELATIONS AND INTERPRETATION OF DATA

All data secured in the survey were placed on punch-cards. It was readily possible to study correlations by mechanical sorting of these cards. A number of such correlations were considered to be essential, for example, the relation between corneal vascularization and riboflavin intake.

The study of food consumption had shown that, as judged by widely accepted recommendations as to proper supplies, the intakes of thiamin and ascorbic acid were very low in most individuals. If these dietary findings can be accepted as indicative of nutritional deficiencies, and this type of interpretation has been widely accepted, physical, dental and special examinations could be expected to reveal related abnormalities, if not particular signs of ill-health. The presence of nutritional anaemia would not be expected from the iron intakes but there was a sufficient number of low intakes of calcium and of riboflavin to have caused physical signs, if, again, the dietary results are indicative of nutritional deficiencies. For the sake of clarity various correlations will be presented in connection with particular nutritive values.

(1) *Calories*

It is frequently assumed that body weight is dependent, at least in normal individuals, upon two factors: calorie intake and energy expenditure. For a proper comparison of the effect of calorie intake on weight it would be necessary to know the energy expenditure. Since it was not possible to determine the latter, recommended daily allowances of calories (selected for sex and age) were used as standards; these standards are, of course, based on average energy requirement. The percentages of these standards, shown as secured in the dietary records, were calculated and comparisons with weight, again expressed in terms of a commonly used standard, were made. Thirty per cent of the girls had weights below normal and three-fifths of this "underweight" group had calorie intakes which were above 90 per cent of the standard. In the case of the boys, 22 per cent were judged as underweight and one-half of these had excellent supplies of calories. There was little apparent relation between calories and body weight, when both of these were graded by standard tables.

(2) *Protein*

Only 4 per cent of the girls and 2 per cent of the boys had a protein intake which would be considered to be low. Evidences of protein deficiency would not have been expected in this group and none were found. Gradings of protein intake and of nutritional status, as determined by physical examination, were compared and reasonably good correlation was found. However, there were only a few individuals with either a low protein intake or who were classed as having a poor nutritional status, so that the apparent correlation can only be considered as partial.

(3) *Calcium*

The following comparisons have been made: calcium intake and number of active dental caries, calcium intake and number of dental fillings, calcium intake and height. No correlation was found in any of these comparisons. One half of the group having the greatest number of active caries had excellent supplies of calcium; the same lack of correlation was true in the case of filled cavities. If the study of food consumption can be interpreted as an indication of food habits, prevalent for some time, an excellent intake of calcium did not prevent the development of caries. A comparison was also made of the dental conditions in those students who were taking an extra source of vitamin D with those without such a supply; no protection against caries was evident. Similarly, no correlation was found between the intake of calcium and height in the group as a whole; approximately 40 per cent of the students who were below normal in height had excellent supplies of calcium. About 20 per cent of those who were above normal in height had poor intakes of calcium.

(4) *Iron*

Theoretically, a relation between iron intake and haemoglobin might be expected. Several investigators, notably Davidson, Fullerton, Howie, Croll and

Orr (11), have reported a lack of correlation. In the East York group there were few low iron intakes and no low haemoglobins. Perhaps this should be regarded as cause and effect but this conclusion is questionable. If a considerable number of the students had been low in iron, or in haemoglobin, a true comparison could have been made. It should be pointed out that 12 per cent of the girls had poor supplies of iron; these all had high haemoglobin values.

(5) *Vitamin A*

Several effects of vitamin A insufficiency have been described: poor dark-adaptation, the development of opacity and thickened areas in the conjunctiva, rough and reddened skin, and increased susceptibility to infection, particularly of the respiratory tract. Information regarding these was secured in the study. Only 6 per cent of the girls and 3 per cent of the boys had low intakes of vitamin A; in fact, most of the students had supplies considerably in excess of the recommended allowance. Evidence of vitamin A deficiency could, then, be expected in only a few persons. No poor dark-adaptation was found in any of the group. On the other hand, 44 per cent of the girls and 59 per cent of the boys showed definite evidence of conjunctival changes, believed to be indicative of vitamin A deficiency. Indeed, about one half of the students who were taking extra vitamin A in a fish liver oil had thickening of the conjunctiva. In view of the current interest in the use of biomicroscopic examination of the conjunctiva for the possible detection of vitamin deficiency, the comparison between vitamin A intake and conjunctival changes is given in Table V. A previously mentioned

TABLE V

CORRELATION OF VITAMIN A INTAKES AND CONJUNCTIVAL CHANGES

Figures given are percentages of total number of each sex.

		Conjunctival Changes			
	Vitamin A Intake	*None*	*Slight*	*Moderate*	*Marked*
Girls:	Excellent	12.2 p.c.	39.1 p.c.	28.3 p.c.	7.0 p.c.
	Good	0.4	1.1	2.4	0.4
	Fair	0	0.7	2.1	0.7
	Poor	0	2.4	2.4	0.7
Boys:	Excellent	5.4	29.2	27.7	22.3
	Good	0	3.5	3.1	1.2
	Fair	0.4	1.9	1.2	1.2
	Poor	0	0.8	2.3	0

explanation of the apparent discrepancy could be advanced, that the records of food consumption for one week do not give a reliable picture of food habits. However, in a recent survey of middle-class families in Toronto (3), fairly adequate supplies of vitamin A were found; even in low-income Toronto families there was no appreciable dietary deficiency of this vitamin (3). All available information indicates that vitamin A intakes are likely to be satisfactory in the Toronto area. Another possible interpretation of the discrepancy between vitamin A intake and the occurrence of conjunctival changes is that an examination of the conjunctiva may not provide a reliable index of vitamin A deficiency.

In one respect there appeared to be a relation between the vitamin A intake

and the incidence of colds reported by the students. Of those who stated that they had had very few colds or none in the past year, 74 per cent of the girls and 69 per cent of the boys had excellent supplies of vitamin A. However, in the group of students who reported frequent colds, 90 per cent of the girls and 82 per cent of the boys had excellent intake of vitamin A. Here again, the point may be raised as to whether the dietary record is a reliable index of nutritive sufficiency; if it is, excellent supplies of vitamin A did not prevent frequent colds in this group of students.

Very few of the students had skin conditions which might be ascribed to lack of vitamin A and, of these, practically all had excellent intakes of the vitamin.

(6) *Thiamin*

If some current conceptions of the effects of suboptimal thiamin intake are correct, most of the examined students should have been depressed and neurotic. Thorough physical examinations failed to reveal any conditions which could be assumed to be due to thiamin insufficiency. Because it is widely assumed that thiamin affects mental processes, it was thought that there might be a relation between academic standing and thiamin intake. The average academic standing of each student for the current year, that is, the one in which this study was made, was provided by the principal of the school and was compared with thiamin intake. Perhaps it could be concluded that there is a correlation since 68 per cent of the girls had academic standings below 70 per cent and 82 per cent of the boys were so graded, while 75 per cent of each sex had low thiamin intakes. However, the few students who had academic grades above 90 per cent also had poor thiamin supplies. Aside from the situation that most of the students had low academic standing and poor supplies of thiamin, which might easily have been a coincidence, there was no evidence of a relationship. It is quite possible that there may be a relationship between thiamin intake and intelligence which would not be revealed by academic standing alone.

In most text-book descriptions of vitamins, anorexia is emphasized as an early sign of thiamin defficiency; it was indeed used as the criterion of thiamin deficiency in a series of experiments designed to determine requirements for the vitamin (12). If this be true, one would expect that a group of adolescents, many of whom had thiamin intakes of less than one milligram per day, during the test period, would show evidence of anorexia by having a small consumption of food. Of those with the smallest supplies of thiamin, 40 per cent of both the girls and boys had food consumption which graded excellent in calories. It might seem possible that there would be a relation between thiamin intake and body weight. More than half of the students who had weights above normal had low intakes of the vitamin.

(7) *Riboflavin*

Two abnormalities are currently ascribed to riboflavin deficiency: cheilosis and corneal vascularization. No cases of cheilosis were seen in this group. Only 7 per cent of the girls and 5 per cent of the boys showed moderate or marked

vascularization. Since over half of the total number had excellent supplies of riboflavin, it might be concluded that the small number of cases of vascularization was due to the reasonably adequate supplies of the vitamin in most of the group. However, 17 per cent of the girls and 24 per cent of the boys had intakes of less than 70 per cent of the standard and practically none of these showed vascularization. Most cases of vascularization were found in students whose dietary records indicated excellent supplies of riboflavin. Statements of the same general nature are equally true for photophobia and lacrimation. It has recently been reported by Sandstead (13) and by Machella and McDonald (14) that there is no relationship between riboflavin intake and corneal vascularization.

(8) *Ascorbic Acid*

Although previous observations in these laboratories (15) and elsewhere had indicated a lack of relationship between ascorbic acid intake and capillary fragility, measurements were made on these students to provide a large set of observations. In this group 75 per cent of the girls and 80 per cent of the boys had calculated intakes of less than 50 mg. of ascorbic acid per day, based on raw food values; it can be assumed, since there was little use of citrus fruits, that the actual intakes were less than 25 mg. per day. This group should have shown, if the dietary results are indicative of food habits, various signs of deficiency of ascorbic acid. No such evidence was found. Two-thirds of the group had normal capillary fragility. There were no cases of marked gingivitis, a condition frequently ascribed to lack of ascorbic acid.

DISCUSSION

The results of the study of food intake, assessed for adequacy in terms of recognized standards, showed widespread insufficiency of thiamin and ascorbic acid, moderate occurrence of deficiencies of calcium and riboflavin, but diets reasonably good in other respects. The use of a "diet score," to give a composite picture, is questionable but, on this basis, 72 per cent of the girls and boys had excellent or good food intakes. It is worth noting that the examining physicians considered that 78 per cent of the girls and 77 per cent of the boys had a nutritional status which was either excellent or good. This may seem to be good correlation; an examination of the detailed comparison, as given in Table VI,

TABLE VI

CORRELATION OF GRADING OF FOOD INTAKE WITH NUTRITIONAL STATUS AS JUDGED BY PHYSICAL EXAMINATION

Figures given are percentages of total number of each sex.

		Grading of Food Intake			
	Nutritional Status	*Excellent*	*Good*	*Fair*	*Poor*
Girls:	Excellent	8.4 p.c.	29.7 p.c.	10.8 p.c.	3.1 p.c.
	Good	4.5	13.6	6.3	1.8
	Fair	2.7	11.1	4.5	1.8
	Poor	0.4	0.7	0.4	0
Boys:	Excellent	7.7	41.2	13.8	1.9
	Good	0.8	7.7	3.5	0.4
	Fair	2.7	11.2	7.7	0.4
	Poor	0	0.4	0.4	0.4

shows some discrepancies. Nevertheless, some correlation is evident. This may tend to strengthen faith in the results of the dietary examination.

The general results of this survey may be summarized as follows:

1. While the intakes of thiamin and of ascorbic acid appeared in most cases to be inadequate (on the basis of current standards), on the whole the food supplies were reasonably good.

2. As judged by thorough physical examination, the nutritional status and the health of most of the students were good.

3. While no cases of gingivitis were seen, dental conditions were definitely unsatisfactory.

The attempts to correlate the various findings, except in a few respects, have been unsatisfactory. There appears to be a connection between the average adequacy of the food consumption and the general level of health, the latter not having been influenced apparently by the low intakes of thiamin and of ascorbic acid. The failure to find any abnormal dark adaptation may have been due to the excellent intake of vitamin A, or it may be a coincidence, but it is difficult to reconcile the marked amount of conjunctival thickening with either or both of the other findings. There was no correlation between calcium intakes and dental conditions. The considerable number of discrepancies has made necessary a consideration of the value of the methods used.

Considerable doubt may be cast upon the method of securing dietary records and on their interpretation. Admittedly, it would have been better to attempt to secure a weighed record; previous experience has shown that this is expensive and decreases the number of co-operative subjects. More serious than this criticism is the question as to whether a record for one week provides a reliable picture of food habits. Should one conclude that a person is deficient in thiamin if a record for one week shows a low intake? Evidence at present available does not appear to be sufficient to answer this question and further investigation on this point is under way in these laboratories. The conclusion that a record for one week is a reliable index of dietary adequacy had been assumed in previous publications by some of us (3) and has been very widely used elsewhere; data based on this conclusion have provided a great deal of the impetus for current nutrition programs.

Another aspect of the interpretation of dietary records is the assessment of adequacy in terms of standards or recommended allowances. Assuming for the moment that a reasonably reliable picture of food habits was secured in this survey, most of these students had supplies of thiamin and of ascorbic acid which would be judged, by current standards, as being inadequate even on a minimal maintenance basis. Since no effects of such deficiencies were apparent, by the methods used, the question may properly be raised as to whether the standards are not too high, or the methods of appraisal not sufficiently sensitive. Current recommendations for thiamin do not take into consideration the amount of dietary fat; animal experiments have clearly shown that dietary fat has a sparing action on thiamin requirements. On the average these students secured about 45 per cent of the total calories from fat; possibly the thiamin requirement is less

because of the amount of fat. Various conclusions may be drawn, any or all of which may prove to be correct: (1) the dietary records did not give a reliable assessment of nutritional conditions, (2) the standards in current use are too high in several respects, or (3) methods of appraisal used in this study were not sufficiently sensitive. The general level of health found in these students was such as to give particular emphasis to the second explanation; reconsideration of nutritional standards is urgently needed. Mention should also be made of probable individual variation in nutritive requirement, a factor which may be responsible for some discrepancies.

It will be noted that no determinations of amounts of vitamins in blood or urine specimens were made. Failure to do so was largely due to a lack of confidence in such procedures. Doubt may be expressed with regard to the validity of interpretation of amounts of vitamins found in single specimens of blood, particularly with regard to ascorbic acid, when the specimens are taken at different times of the day, regardless of meals. It might have been advisable to obtain fasting blood specimens; unfortunately this was not possible in this group of subjects.

Experience derived from this survey has led to the conclusion that the assessment of nutritional status in a large group is at present difficult, if not impossible, when definite clinical signs of deficiencies are absent. Tentatively it may be suggested that dietary records are useful and that physical examination is essential. The value of several specific tests is doubtful. There is urgent need for the development of reliable methods for nutritional examinations, particularly for what have been described as subclinical states.

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Bacterial Food Poisoning*

PART II†

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STAPHYLOCOCCUS FOOD POISONING

Incidence

The commonest, and in some respects the least controllable form of food poisoning is that due to enterotoxin-producing staphylococci. Its actual incidence is unknown, since the nature of the syndrome is such that outbreaks are only likely to be reported when fairly large groups are involved; while many health authorities, particularly in Great Britain, have been either unfamiliar with the condition or sceptical of its existence. But the ubiquity of staphylococci, and the lack of any evidence pointing to a specifically limited distribution of enterotoxigenic strains; the great variety of foodstuffs in which the enterotoxin is known to have been elaborated; and the prevailing ignorance and apathy respecting the prevention of air, milk, finger, droplet or fly-borne contamination of foodstuffs, make a high incidence of staphylococcal food poisoning inevitable. That the incidence is not still higher may be explicable in terms of recent findings in these laboratories, now to be reported, which indicate that among staphylococci isolated from various sources, food poisoning strains may not comprise as high a percentage as other workers have claimed.

Control of Milk-borne Outbreaks

From the first, the importance of milk and milk products as vehicles for staphylococcus food poisoning has been recognized. This is no doubt largely due to the considerable handling such foodstuffs undergo prior to consumption; to their suitability as media in which staphylococcal pullulation and enterotoxin production may occur, without any signs of resulting spoilage; and to the apparent eagerness of the public to see pastries and confections displayed in warm shop windows. But another important factor is the frequency of endogenous infection of milk with staphylococci, against which the elimination from dairy herds of cows with infected udders provides only a partial safeguard. Gwatkin (37),

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Minett (38), and others have reported that the milk of cows showing no mastitis, or any other abnormality, may contain enterotoxigenic staphylococci; and a striking illustration of this fact has been given earlier in this paper. Although the origin of such staphylococci, and their significance to the cow harbouring them, is not clear, these findings suggest an analogy to the situation described by Wilson (39, 40) in which human naso-pharyngeal carriers caused a series of outbreaks of food poisoning. Perhaps an even closer analogy may be traced in the recent report of Duncan and Walker (41), who found *Staphylococcus aureus* in the milk of a high proportion of healthy nursing mothers. Unfortunately no attempt was made to determine whether the strains were potentially enterotoxigenic; but the majority of the infants remained healthy, although staphylococci were in many instances isolated from their throat and rectum. Typical staphylococcal food poisoning due to human milk is of course too remote a contingency to warrant serious discussion. However, it is noteworthy that staphylococci were isolated from the diarrhoeal stools of six newborn infants, one of whom died from "marasmus and dehydration": a finding which supports a suggestion of Dolman (42) that staphylococci might occasionally establish themselves in the intestinal tract in sufficient numbers to produce a dysenteric syndrome through elaboration of enterotoxin *in situ*.

Dolman (42, 43) has emphasized that pasteurization markedly reduces the food poisoning hazard from endogenous staphylococcal infection of milk. Prompt, maintained cooling is an important supplementary precaution. Pasteurization alone may fail as a safeguard, owing to staphylococci having survived an inefficiently carried out process; or through the heat-stable enterotoxin having been elaborated during improper storage of the milk prior to pasteurization, as in the outbreak recently recorded by Caudill and Meyer (44). Moreover, pasteurization will not prevent outbreaks due to subsequent exogenous contamination. Staphylococci introduced exogenously into milk or a milk product can be held from elaborating significant amounts of enterotoxin by refrigeration of the foodstuff immediately after its preparation, as well as of the ingredients prior to their admixture. In fact, Stone (45) has claimed that failure to cool milk promptly, or to maintain it and other ingredients of dairy products at a low temperature, is mainly responsible for staphylococcal food poisoning outbreaks of this type. Another precautionary measure worthy of mention, apart from pasteurization and refrigeration, is the rebaking of chocolate éclairs, and other pastries containing custard or cream fillings, to whose feasibility Stritar, Dack and Jungewaelter (46) first drew attention. Such procedures are designed to cope with the special liability of pastries to be exposed to warm room temperatures after manufacture, but cannot be effective against enterotoxin already elaborated in some ingredient prior to its incorporation in the final foodstuff.

Control of Exogenous Contamination

Cow's milk is the only significant source of endogenous staphylococci in a foodstuff, all other contamination by these organisms being exogenous: a fact which eliminates certain difficulties inherent in the control of Salmonella food infection. But the presence of staphylococci in room air, as well as on healthy

human skin and mucous membranes, makes prevention of exogenous contamination of foodstuffs therewith extremely difficult; and speculation as to the possibility of altogether eliminating such contamination soon exhausts itself in devising obviously impractical measures. For not only would food handlers have to be always well scrubbed, and their hands free from pimples and abrasions, but they should also be certified not to carry enteroxigenic staphylococci in their nose and throat. Moreover, they should always wear freshly-laundered caps and gowns, their utensils should be thoroughly scalded before and after use, their kitchens should be fly-proofed, and the air filtered or sterilized. Thus installed in their operating theatres, these ideal food handlers would provide further touches of security for the proceedings by using aseptic technique. There would be no rubbing of eyes, scratching of hair, or fingering of nose; and the food would not be coughed or sneezed into, sweated upon, talked at, or wept over. A visit to the kitchen of almost any contemporary eating-place will reveal what counsels of perfection such proposals represent.

Impossible though it may be to follow such a regimen in the institutional, restaurant or household kitchen, some nearer approach to it than is now customary is quite feasible. The trend towards more group or community feeding, which the war has accentuated, makes imperative an intensive educational program for health officials (sanitary inspectors in particular), for food handlers, and for the public at large, regarding staphylococcal food poisoning and its prophylaxis. This education is necessary not only to reduce exogenous contamination of foodstuffs, but also as a precursor to passage and enforcement of such legislative measures as the pasteurization of milk and milk products, the refrigeration of foodstuffs, and bans upon the display of meats and pastries in shop windows, which must supplement the individual efforts of more enlightened food handlers if the incidence of this type of food poisoning is to be brought really low. The educational and legislative efforts alike should be especially directed against the still prevailing notion that foods are safe until signs of spoilage appear. Enormous numbers of staphylococci may be present in a food without changing its appearance, odour, or flavour.

Effect of Time and Temperature on Enterotoxin Production

Rigorous confinement to the refrigerator, rather than casual disposition on the shop counter or in the kitchen cupboard, must be applied not merely to the "perishable" foodstuffs, because of their suitability as a growth medium for putrefactive organisms as well as staphylococci, but also to such commodities as ham and cheese, whose keeping qualities encourage frequent handling and laxity in storage. If a food contaminated with enterotoxigenic staphylococci be only intermittently placed in the refrigerator, and is at other times exposed to warm temperatures, the organisms may elaborate sufficient enterotoxin to render the food poisonous. Kelly and Dack (47) report, for instance, that two human volunteers had a very severe attack of staphylococcal food poisoning following consumption of 48 gm. of ham and 63 gm. of bread respectively, which had each been inoculated with an enterotoxigenic strain, and were then incubated for 5 hours at 37°C., refrigerated for 16 hours, and reincubated for another 6 hours.

Under natural conditions of food preparation, exposure to incubator temperature must be rare. But the air temperature of the kitchen is often uncomfortably high; and it may here be recalled that Barber (48) in his now classic paper, reported having made himself ill by drinking a mixture of milk and cream which had been stored for 5 hours at 28-30°C. Even at the average temperature of a warm room, say 20-23°C., enterotoxin production can in a surprisingly short period reach a level of potency sufficient to cause food poisoning in man. For instance, Dolman (48) describes a small outbreak due to vanilla slices, containing custard filling and coated with sugar icing, in which there was apparently a maximum exposure of the food to room temperature for 14 hours prior to consumption; while Kelly and Dack (47) allude to an outbreak due to ham sandwiches which had been kept 18 hours at room temperature. Again, an account will be given below of an outbreak in which ham, after a maximum exposure to warmth of 10 hours, was the peccant food. Each of these outbreaks occurred during the summer months, when the average temperature to which the foods were exposed may have exceeded 22°C. But that enterotoxin can be elaborated at this temperature was definitely shown by Minett (38), who kept raw, pasteurized and sterilized milk, inoculated with an enterotoxigenic strain, for 2 days at 22°C., and then obtained a positive reaction on intraperitoneal injection of 5-10 cc. of the boiled materials into kittens. Similar results were given by the artificially-inoculated cream filling of a layer cake, which was stored for 3 days at 22°C. before being tested. Finally, in this laboratory, in an experiment to be described more fully later, as little as 10 gm. of a sausage inoculated with a potent strain, and kept at 22°C. for 52 hours, caused a violent reaction in a human volunteer.

Such observations help to explain the comparative frequency with which ham is involved in staphylococcus food poisoning outbreaks. Enough examples of such outbreaks have come to our attention at the Provincial Laboratories in Vancouver in recent years to suggest that this commodity is subject to special contamination hazards, not only from the frequent handling to which it is liable after purchase, but also perhaps because present methods of curing and "tenderizing" are at times unsatisfactory from the public health standpoint. Incidentally, the only fatality from staphylococcal food poisoning known to have occurred in Canada, followed the consumption, by a man in Ottawa, of the remains of a ham which had been kept for 4 days in a drawer at a rooming house. The following account of an outbreak may serve to emphasize the special importance of educating food handlers in the Armed Forces respecting the prophylaxis of food poisoning due to ham.

A group of 53 soldiers fell ill in the course of regimental manoeuvres on a hot summer day, the symptoms being acute vomiting and diarrhoea, with severe cramps and marked prostration. Many had a temperature of around 100°, while in one instance the temperature went to 104°. Some of the men were so ill that it was feared they might not reach hospital alive. However, most cases began to recover a few hours later, and the majority were well next day; but a few remained in hospital for up to 10 days. The attacks began shortly after supper, which was taken at 5 p.m. The previous meal had been at noon. Each meal comprised the same foods, including sliced ham, which was served out from a closed, double-jacketed aluminum container into which it had been packed by hand that same morning. The

ham had been boiled the previous evening, and was placed in the cooler at 45°F. overnight. At about 7 a.m. it was cut and the slices packed into the container. The container was carried in a covered vehicle, and was not openly exposed to the sun except when the ham was being served at each meal. But shortly before the ham was packed into it, the container had been scalded, and was probably still warm when sealed; whereafter, being constructed on air-tight thermos principles, it would have provided the requisite conditions for enterotoxin production.

Some portions of the ham were examined bacteriologically at the Provincial Laboratories, and yielded enormous numbers of both haemolytic and non-haemolytic staphylococci. None of the colonies showed beta-haemolysis on sheep blood agar. Some coliform organisms were also present.

If it be assumed that contamination occurred at the initial slicing and packing of the ham, only 10 hours were available for enterotoxin production. Additional staphylococcal contamination may easily have been introduced while the ham was served out at lunch, but it is hard to imagine that much enterotoxin could have been elaborated in the 5 hours elapsing between lunch and supper, even although it was a hot day. Thirteen among a total of 66 persons ate portions of the ham without ill effects. Their circulating beta-antitoxin titres were no higher than those of persons known to be susceptible to the enterotoxin.

Clinical Manifestations

The symptomatology in an outbreak of staphylococcal food poisoning is so characteristic that the diagnosis need seldom be in doubt. After a symptomless interval of usually 1-4 hours, followed by a period of nausea which may last from only a few minutes up to nearly an hour, sudden vomiting occurs. Several bouts of vomiting may ensue over the next few hours, and there may also be abdominal cramps and marked diarrhoea. The pulse is rapid and in severe cases may become thready, the temperature is often raised, and the patient may be collapsed and apprehensive, with numbness of the extremities, pallor, and cold sweats. After 3-5 hours, and sometimes earlier, improvement sets in, colour returns, the pulse becomes stronger, and a desire for food and drink may be expressed. Often, however, for a day or two and sometimes longer, anorexia may persist, with nausea, diarrhoea, or abdominal discomfort, and considerable loss in weight. The mortality rate from staphylococcus food poisoning is certainly very low, but a few apparently authentic fatalities are on record; while Kodama, Hata and Sibuya (49) have reported a premature birth, with death of the infant, following an attack due to fish-sausage.

Personal experiences based on ingestion experiments with the enterotoxin leave one in no doubt as to its very grave, and possibly lethal effects, if a sufficiently large dose were taken. It is now accepted that the action of the enterotoxin is not directly upon the gastro-intestinal tract, but rather upon some specific area of the central nervous system. The nature of the stimulus, and the exact location of the susceptible centre, are unknown;* but it is not difficult for those familiar with the action of the enterotoxin to envisage a fatal degree of shock. A few words of caution seem pertinent here for the benefit of any who may contemplate ingestion experiments. When filtrates are prepared from appropriate

**However, Bayliss (74) has presented important evidence as to the probable pathways of the reflex, and the location of the centre, involved in the feline vomiting reaction induced by enterotoxin.*

strains by some modification of the soft agar-partial CO_2 atmosphere method, first advocated for enterotoxin production by Dolman (50), as little as 1 cc. taken by mouth may evoke a severe reaction in man. Therefore for human volunteers, the first dose of a filtrate prepared from a new strain should not exceed 0.5 cc. Moreover, following negative reactions, increments between successive doses should be small; for the margin between marked positive and completely negative reactions in the same individual may be as little as 0.5 cc., or even 0.25 cc. Again, if a volunteer of unknown susceptibility is being used, the dose of a new filtrate under test should not be increased beyond 2 cc. until other volunteers (including, if possible, at least one of known susceptibility) have taken similar amounts of the material. This precaution covers the possibility that a relatively insusceptible person might otherwise take such large amounts of a potent filtrate that the mechanism determining his resistance (*vide infra*) is suddenly overcome, with dire consequences.

A few additional comments upon the subjective aspects of the syndrome seem warranted. These observations are based on 16 severe positive reactions shared among 6 laboratory colleagues during the past year, 3 of whom underwent the experience 4 times. Transient attacks of nausea, of increasing duration and severity over a period of ½-1 hour or even longer, their first onset sometimes being noted in less than an hour after ingestion of a filtrate, may foretell an impending full-fledged reaction. Alternatively, eructations, increased salivation, itching and running of the nose, sneezing and occasionally headache or dizziness, may be noticed before the onset of definite nausea. Eventually, at a time ranging in this series from 83 minutes to 4½ hours after ingestion of the test material, an especially acute attack of nausea ends in an explosive and utterly uncontrollable bout of vomiting. Following the loss of the greater part of one's last meal in this fashion, there is temporarily a sense of relief; but further bouts of vomiting occur, each preceded by a brief paroxysm of nausea. The intervals between vomiting were in some instances initially as short as 7 minutes, but later became more prolonged, two hours elapsing in one case between the penultimate and the final vomiting. Over the whole series, there was a range of from 4 vomitings in 1¾ hours, to as many as 16 vomitings in a period of nearly 12 hours. The vomiting is accompanied, and sometimes replaced by, considerable retching. At first one is astonished to note from what depths, and after what strivings, it is possible to recall portions of meals long forgotten; but as the stomach empties of food, bile-stained and even blood-flecked mucus becomes the chief return. Abdominal cramps and diarrhoea are often very marked, and may precede the vomiting, but more usually begin after the vomiting has started, and may continue after it has ceased. The first evacuation may be merely a loose stool, but this is apt to be followed by frequent and remarkably profuse discharges of watery fluid. The resulting dehydration no doubt accounts largely for the pinched, shocked appearance of the subjects; and to some extent also for the marked lassitude and depression which sets in after the first glamour of a positive reaction has given way to disenchantment. The ordeal can perhaps be compared to pregnancy, in that it may have been light-heartedly enough embarked upon the first time, but is not so willingly repeated; and in its being made bearable, as the situation moves towards a climax, only by the reflection that deliverance is bound to come. In

one or two instances, a degree of exhaustion set in which bordered on collapse; but recovery always commenced before the subjects were too far gone to record the times or recount the details of their various episodes. Once begun, recovery was usually rapid, the only after-effects being a large appetite, and a sense as of calm after a storm. But sometimes intermittent nausea and anorexia, and more particularly diarrhoea, persisted for several hours, and on a few occasions for about two days, after the last vomiting.

Reference has already been made to the narrow margin between a dose giving no reaction at all, and one giving a severe reaction of the type described above. However, border-line reactions do occur. For instance, in one experiment a volunteer had increasing nausea for 1½ hours, starting nearly 3 hours after taking a dose of filtrate, then vomited once, and had no further symptoms save for a few mild abdominal cramps. On other occasions, subjects complained of feeling seedy and nauseated for several hours, without having actual vomiting or diarrhoea. Mild attacks of staphylococcal food poisoning, characterized by symptoms such as these, must be very common.

Hypothetical Relationship between Enterotoxin and Beta-toxin

Earlier reports on the food poisoning role of staphylococci were liable to be greeted with incredulity, but lately there has developed a tendency to believe this organism capable of performing almost any wonder. Following the report of Dolman, Wilson and Cockcroft (51) on the use of the kitten as test animal for enterotoxigenic strains, various workers used this method as a basis for surveys into the incidence of such strains among staphylococci from different sources; while others evolved special methods of identifying enterotoxigenic strains, whose validity rested upon an alleged parallelism between certain *in vitro* properties of a culture, and the kitten reaction given by its filtrate. The results of these surveys suggested that a high percentage of strains of human or bovine origin, and also of strains isolated from foods, were potentially enterotoxigenic. Fresh attention was drawn by Gwatkin, Hadwen and LeGard (52) to the frequency with which alpha-toxigenic staphylococci might be isolated from cow's milk, particularly when mastitis was present; while the special association of beta-toxigenic strains with cow's milk was pointed out by Minett (53) and by Bryce and Rountree (54). The alpha-toxin had been shown clearly enough by Dolman (50) to be distinct from the enterotoxin, but the data differentiating the latter from the less well known beta-toxin were perhaps not so convincing. Alternatively, the natural desire of the human mind for simplicity may have led to a close association being conjectured between the beta- and enterotoxins. In any event, evidence began to accumulate which was interpreted by various workers as pointing to such an association. For instance, Kojima and Kodama (55) found difficulty in differentiating the enterotoxin from beta-toxin present in crude staphylococcal filtrates. Using the kitten test as a criterion of the presence of enterotoxin, they failed to isolate any non-enterotoxigenic strain which produced either the combined alpha- and beta-toxins, or the beta-toxin alone. Moreover, in attempting to purify the enterotoxin in this laboratory, Darrach (56) finally obtained a powder of which as little as 0.025 mgm. dissolved

in 1 cc. of physiological saline gave a positive kitten reaction. This solution, however, contained about 10 hot-cold lytic units for a 1 per cent sheep erythrocyte suspension, which could not be separated from the factor causing the kitten reaction. Shortly afterwards, Woodward and Slanetz (57) and also Wilson and Fulton (58) independently reported similar difficulties in separating the beta-toxin and enterotoxin, and suggested the possibility that they were the same entity.

There were numerous arguments against this hypothesis. First, the staphylococcal strains isolated from incriminated foods often fail to produce beta-toxin, as in the two outbreaks described earlier in this paper. Food poisoning strains may, in fact, be altogether non-haemolytic. Secondly, in some early experiments reported by Dolman (50), several volunteers drank up to 15 cc. of a filtrate from strain "24" (a potent beta-toxigenic strain) without any ill effects. Thirdly, Dolman and Wilson (59) adduced serological evidence, based on flocculation procedures, which pointed to the separate nature of the beta- and enterotoxins; and referred to a strain which was alpha- and beta-toxigenic, but not enterotoxigenic. Finally, the frequency with which beta-toxigenic strains may be found in raw cow's milk would entail, assuming that these were necessarily enterotoxigenic strains, a much higher incidence of milk-borne staphylococcus food poisoning than can be held to prevail, even after allowances have been made for the non-recognition of many outbreaks.

Enterotoxin and Beta-toxin Differentiated by Ingestion Experiments on Man

A request for help in the testing of strains of staphylococci and other organisms isolated from food poisoning outbreaks, received from Prof. G. S. Wilson, Director of the Emergency Public Health Laboratory Service in England, provided the incentive for reinvestigation of the specificity of the kitten vomiting reaction, and for a crucial test of the hypothesis that the beta- and enterotoxins might be identical. Conclusive evidence was obtained that the enterotoxin is entirely distinct from the beta-toxin, but that both entities may evoke kitten reactions.

Among the cultures received for investigation from Prof. Wilson, and from his colleague, Dr. Forrest Fulton, was staphylococcus strain 12069, originally isolated from brawn (head cheese) which had been epidemiologically implicated in an outbreak of severe gastro-enteritis affecting 32 persons. This strain originally produced a potent alpha-toxin, but no beta-toxin. Unboiled filtrates had given positive kitten reactions, but boiled filtrates had proved inert. On arrival in Canada, after several weeks in transit, the culture was found to be unstable. When first plated out on sheep blood agar, the resulting growth showed *Staphylococcus aureus* with alpha-toxigenic colonies predominating; but numerous colonies had developed the wide zone of modified haemolysis characteristic of beta-toxin. When an alpha-toxigenic colony was picked, suspended in nutrient broth, and replated, a small proportion of the resulting colonies were again beta-toxigenic variants. Eventually, after many subcultures, a stable alpha-toxigenic strain was recovered. The variant colonies, on the other hand, always bred true.

Filtrates were prepared by the soft agar-partial CO_2 atmosphere method from both the parent strain and its variant, hereafter to be designated 12069 alpha and 12069 beta respectively. Filtrate 12069 alpha contained 500 units of rabbit cell lysin (alpha-toxin) per cc., and showed 40 units of sheep cell lysin at 37°C., with no additional lysis when titrations were placed in ice-water at 10°C. If any beta-toxin were present, the amount was therefore negligible. On the other hand, filtrate 12069 beta contained 300 haemolytic units of alpha-toxin per cc., and no less than 16000 units of hot-cold sheep cell lysin (beta-toxin) per cc. These filtrates were kept in the refrigerator, and were used for numerous human feeding and feline injection experiments, representative examples of which will now be recorded.

Since the fortuitous production of the variant by this culture offered so obvious an opportunity of determining definitely whether or not enterotoxigenic capacity had been affected by the acquisition of the power to produce beta-toxin, it was decided to resort directly to human volunteers. In Table I are set forth the results obtained by feeding one's least pusillanimous associates with various amounts of filtrates 12069 alpha and beta. A filtrate prepared from strain J 32, which produces no alpha-toxin, but is a fairly good beta-toxin producer, was also taken by several persons. No reactions whatever followed ingestion of up to 10 cc. amounts of 12069 beta and J 32 filtrates, although many of those taking them proved susceptible to far smaller volumes of 12069 alpha filtrate. For instance, 1 cc. of the latter filtrate caused a severe reaction in 4 persons, 2 of whom had been unaffected by previous doses of 0.5 cc. and 0.75 respectively. Two other subjects, who were unaffected by 1 cc. amounts, had severe reactions from 2 and 3 cc. respectively. One subject (N.B.) took 1, 2, 3, 4 and 5 cc. amounts on successive days, all without effect. These results indicate that strain 12069 alpha, which produces no beta-toxin, is nevertheless an excellent producer of enterotoxin; and that its beta-toxigenic variant produces no enterotoxin. Likewise, beta-toxigenic strain J 32 shows no evidence of being enterotoxigenic.

Further verification of the separate identities of the beta- and enterotoxins was afforded by titration of the beta-antitoxin content of the sera of persons involved in the ingestion experiment described above. Subject H.C., who reacted markedly to 1 cc. of filtrate 12069 alpha, had a higher titre of circulating beta-antitoxin than had subject N.B., who proved insusceptible to 5 cc. of the same filtrate; and there were other inconsistencies of the same order. These findings are in accord with the lack of parallelism noted between beta-antitoxin titres and immunity to food poisoning in the ham outbreak earlier described. Again, subject C.E.D., who failed to react to 1 cc. of the 12069 alpha filtrate, but showed a marked reaction to 3 cc., had a definitely positive, though mild, reaction two months later from taking a mixture comprised of 1.5 cc. of the same filtrate and 0.75 cc. of a horse beta-antitoxic serum prepared against J 32 toxin. This amount of serum was selected on the basis of being fully sufficient to neutralize the large amounts of beta-toxin contained in 1.5 cc. of the 12069 beta filtrate.

Subject N.B., who resisted 5 times the dose causing a marked reaction in several other persons, may be regarded as representative of that relatively small percentage of apparently immune persons who suffer no ill effects in outbreaks

of staphylococcal food poisoning. In an attempt to determine whether his resistance might be correlated with a high titre of specific circulating antitoxin, subject C.L.A., who had reacted markedly to 2 cc. of filtrate 12069 alpha 4½ months previously, took a mixture comprised of 2 cc. of the same filtrate and 8 cc. of subject N.B.'s serum. The mixture was held at 37°C. for 20 minutes, and

TABLE I

HUMAN REACTIONS TO INGESTION OF STAPHYLOCOCCUS FILTRATES

Filtrate	Subject	Volume Taken	Reactions
		cc.	
C 12069 (parent): *alpha-toxin and enterotoxin*	L.E.R.	0.5	Nil
	R.R.	0.75	
	C.L.A.	1	
	C.E.D.	1	
500 rabbit cell units, 40 hot sheep cell units, and no additional hot-cold sheep cell units per cc.*	N.B.	1-5	
	H.C.	1	Nausea at 2½ hrs.; vomited 6 times from 4-7 hrs.; diarrhoea ++++ from 5½-9½ hrs. Rapid recovery after 10½ hrs.
	R.L.-H.	1	Nausea at 2¾ hrs.; vomited 6 times from 3½-6 hrs.; cramps +++. Rapid recovery after 6½ hrs.
	L.E.R.	1	Nausea at 3 hrs.; vomited 4 times from 3¼-5 hrs.; diarrhoea at 5½ and 8½ hrs. Rapid recovery after 5½ hrs.
	R.R.	1	Nausea at 3½ hrs.; vomited 7 times from 3¾-6¼ hrs.; diarrhoea ++ from 4½-6¼ hrs. Rapid recovery after 7 hrs.
	C.L.A.	1	Nausea at 1¾ hrs.; vomited 9 times from 2¼-10½ hrs.; diarrhoea at 5¼ and 7¾ hrs. Anorexia next day, then rapid recovery.
	C.E.D.	3	Nausea at 2¼ hrs.; vomited 5 times from 2¾-6¼ hrs.; diarrhoea ++++ from 2¾-5 hrs. Rapid recovery after 9 hrs.
C 12069 (variant): *alpha-toxin and beta-toxin*	N.B.	3	Nil
	D.G.B.M.	4	
	R.J.W.	5	
300 rabbit cell units, 120 hot sheep cell units, and 16,000 hot-cold sheep cell units per cc.	R.L.-H.	8	
	C.L.A.	10	
	C.E.D.	10	
J 32: beta-toxin only	E.T.	1	Nil
	M.D.T.	1	
	H.C.	2.5	
No rabbit or hot sheep cell lysins; and 1000 hot-cold sheep cell units per cc.	R.L.-H.	2.5	
	L.E.R.	2.5	
	C.L.A.	10	
	C.E.D.	10	

Subjects C.L.A., H.C., E.T., and M.D.T. were young women.

*Rabbit cell units = inverse of dilution giving 50 per cent lysis of 1 per cent rabbit erythrocyte suspension after 1 hour of incubation in water bath at 37° C. in physiological saline.

Hot sheep cell units = inverse of dilution giving 50 per cent lysis of 1 per cent sheep erythrocyte suspension in physiological saline, after 1 hour of incubation in water bath at 37° C.

Hot-cold sheep cell units = as above, but after 1 additional hour of immersion in ice-water at 10° C.

imbibed in chocolate milk, which effectively absorbed any cannibalistic taint. A typical severe reaction ensued. Apparently the resistance of subject N.B. cannot be ascribed to specific circulating antibodies. An unusually phlegmatic vomiting mechanism, and a gastro-intestinal tract relatively impermeable to the enterotoxin, seem reasonable alternative explanations. In neither event would it be safe to assume that such a person could continue to take increasing dosages without risk of a heavy dose being on some occasion absorbed, with disastrous effect.

Whether natural specific immunity to enterotoxin actually exists in man is a point which remains undetermined. Since alpha- and beta-antitoxins are present in most human sera, sometimes to quite high titres, circulating anti-enterotoxin might also be expected to occur, provided of course that the enterotoxin were antigenic. The consensus among workers in this field to date has been that the enterotoxin was antigenic; but this question needs further examination. Woolpert and Dack (60) appear to have induced active immunity in monkeys, but the serum of such animals did not confer passive protection upon other monkeys; and no data are yet available to indicate whether the susceptibility of human beings to the enterotoxin can be lessened by a series of parenteral injections of the toxoided material. Several successive doses of enterotoxin by mouth certainly do not evoke any significant immunity response. Table II shows

TABLE II

LACK OF IMMUNITY DEVELOPMENT FROM SUCCESSIVE INGESTIONS OF ENTEROTOXIN

Subject	Date	Material Taken	Reaction
R.L.-H.	18.v.1942	1 cc. 12069 alpha	Vomited 8 times
"	21.vii.1942	1 cc. 12069 alpha	Vomited 16 times: diarrhoea ++
"	30.vii.1942	1 cc. 12069 alpha	Vomited 10 times.
"	15.ix.1942	1 cc. strain H	Vomited twice: diarrhoea ++++
C.L.A.	19.xi.1942	1 cc. 12069 alpha + 0.25 cc. Wood (alpha) antitoxin	Nil
"	21.xi.1942	2 cc. 12069 alpha + 0.5 cc. Wood antitoxin	Vomited 9 times: diarrhoea ++
"	12.ii.1943	10 gm. sausage infected with 12069 alpha	Vomited 6 times: diarrhoea +++
"	19.ii.1943	10 cc. extract from sausage infected with 12069 alpha	Vomited 8 times: diarrhoea ++++
"	5.iv.1943	2 cc. 12069 alpha + 8 cc. human serum (N.B.)	Vomited 10 times: diarrhoea ++++
C.E.D.	19.v.1942	1 cc. 12069 alpha	Nil
"	20.v.1942	3 cc. 12069 alpha	Vomited 5 times: diarrhoea ++++
"	22.vii.1942	1.5 cc. 12069 alpha + 0.75 cc. J 32 (beta) antitoxin	Vomited once.
"	21.xi.1942	1.5 cc. 12069 alpha + 0.3 cc. Wood antitoxin	Vomited 5 times: diarrhoea ++++
"	19.ii.1943	10 cc. extract from sausage infected with 12069 alpha	Vomited 6 times: diarrhoea ++++

the reactions of three subjects who each took enterotoxin in some form on four separate occasions over periods ranging from 4-9 months. None of these subjects gave any evidence of the development of immunity during the course of the experiments.

One filtrate prepared from strain 12069 alpha early in May 1942 was used

throughout these experiments, and showed no apparent diminution in its enterotoxin potency 11 months later. This fact accords with the reports of several workers, who have drawn attention to the marked stability of the enterotoxin when kept in the cold. Heat stability has also been generally accepted as a characteristic attribute of the enterotoxin, and the usual method of performing the kitten test presupposes that little enterotoxic potency is lost by heating a filtrate to 100°C. for 20-30 minutes. However, the heat stability of the alpha- and beta-toxins may vary according to the strain of origin, as Dolman (61) has shown; and there is evidence that some degree of variability may also be exhibited by enterotoxins prepared from different strains. According to Dolman and Wilson (59), for instance, filtrates prepared from the Barss strain (whose alpha- and beta-toxins have above-average heat resistance) appeared to lose about half their enterotoxic potency when boiled for 20 minutes. Filtrates of strain 12069 alpha, on the other hand, were reported from England to have given negative kitten reactions after being boiled for 20 minutes: and our own observations on kittens injected with such filtrates confirm this point. The relative heat-lability of the enterotoxin prepared from this strain was further verified by giving 2 cc. of boiled filtrate to subject R.L.-H., who experienced only slight nausea. By contrast, this same person suffered severe reactions, both prior and subsequent to this occasion, after ingestion of 1 cc. amounts of the unboiled filtrate.

Differentiation of Kitten Reactions Due to Enterotoxin and Beta-toxin

The 12069 alpha- and beta-filtrates used in the foregoing human feeding experiments were tested on kittens by intraperitoneal injection. The alpha-toxin in the filtrates was first neutralized by addition of an appropriate amount of a horse alpha-antitoxic serum prepared against Wood toxin. Some 60 kittens of various sizes were used in a series of experiments from which representative examples have been selected and set forth in Table III. Evidently an intraperitoneal injection of beta-toxin may cause early and repeated vomiting, with diarrhoea, followed by death a few hours later. On the other hand, after administration of enterotoxin by the same route, there is a latent period of ¾-2 hours (which approximates in length to that of the human syndrome) before the bouts of vomiting and diarrhoea begin. Diarrhoea is more conspicuous in the beta-toxin syndrome, tenesmus and watery stools often appearing early.

Kittens given the beta-toxin are apt to be restless from the time of injection, appear sometimes to have severe abdominal pain, and show increasing general distress. Then, if a large enough dose has been given, they collapse, and death supervenes typically in from 4-6 hours, or possibly overnight. Following lesser doses of beta-toxin, the animal may survive for a few days, but becomes very emaciated, incontinent and miserable, and then either dies or has to be killed. The autopsy findings, apart from the general emaciation, are usually inconspicuous, but there is often vascular engorgement, which seems to involve particularly the lungs; and occasionally there may be small haemorrhages in the lungs or mesentery. These findings resemble those described by Bryce and Rountree (54) in rabbits killed with beta-toxin.

By contrast, kittens receiving the enterotoxin tend to be somnolent and apathetic, although they may become temporarily restless as their first vomiting

bout approaches. They now appear giddy and nauseated, and may retch, while waves of peristalsis may be noted against the abdominal wall. As soon as each vomiting bout is over, the animals appear considerably relieved, and relapse into lethargy, curling up with heads on paws: an attitude very reminiscent of that adopted under similar circumstances by human volunteers. Once recovery has set in, it usually progresses fairly rapidly, as in man, and by next morning the kittens seem restored to normal. Even after being given enterotoxin on two or three successive days, some loss in weight may be the only apparent deleterious

TABLE III

COMPARISON OF EFFECTS OF STAPHYLOCOCCUS ENTEROTOXIN AND BETA-TOXIN INJECTED INTRAPERITONEALLY INTO KITTENS

Kitten No.	Weight (gms.)	Vol. of Filtrate	Source and Treatment of Filtrate	Reaction
		cc.		
567	330	1	*12069 alpha* (neutralized with Wood serum)	Vomited at 68′, 70′, 80′, 83′, 95′; diarrhoea at 75′.
562	860	1.2	"	Nil
706	515	2	"	Vomited at 46′, 54′, 2¾ hrs; diarrhoea at 1½ hrs.
705	490	3	"	Vomited at 1¾ hrs., 2½ hrs.
704	480	4	"	Nil
569	415	3	*12069 beta* (neutralized with Wood serum)	No vomiting; died overnight.
748	510	2	"	Vomited at 6′, 11′, 29′, 69′; diarrhoea at 3′, 28′, 47′; died in 4-5½ hrs.
701	745	3	"	Vomited at 10′, 35′; diarrhoea at 3′, 21′; died in 4-5½ hrs.
740	1020	3.5	"	Vomited at 4′, 5′, 6′, 11′, 17′, 24′, 39′, 74′; diarrhoea at 3′; died in 6¼ hrs.

12069 alpha filtrates neutralized with Wood serum showed no free rabbit or sheep cell lysins.

12069 beta filtrates neutralized with Wood serum showed no free rabbit cell lysin, but retained 40 hot sheep cell and 16,000 hot-cold sheep cell units per cc.

effect. However, it seems theoretically probable that very large doses of enterotoxin might have serious, and even fatal effects on kittens. Dolman, Wilson and Cockcroft (51) in fact reported occasional fatalities, with no consistent abnormalities at autopsy, following administration of large doses of potent filtrates.

From Table III it is apparent that some kittens may fail to vomit following the injection of amounts of beta- or enterotoxin sufficient to provoke positive reactions in other kittens. For example, although the failure of kitten 562 to react to 1.2 cc. of the enterotoxin may have been due to this being too small a dose for a relatively large (860 gm.) kitten, the same argument cannot be used in the case of kitten 704 (480 gm.), which was unaffected by 4 cc. of enterotoxin. Again, although kitten 569 (415 gm.) died overnight after receiving 3 cc. of beta-toxin, it did not vomit. Yet 2 cc. of the same filtrate sufficed to cause

repeated vomiting in kitten 748 (510 gm.), death occurring a few hours later. Natural immunity to the respective toxins is a possible explanation of such insusceptibilities. In view of the unquestioned antigenic properties of the beta-toxin, some degree of natural immunity may account for the resistance exemplified by kitten 748. But whether natural immunity could explain the resistance shown by kitten 704 depends on settlement of the controversy respecting the antigenicity of the enterotoxin.

An alternative explanation lies in the possibility that at times some portion of a supposedly intraperitoneal injection may inadvertently be given subcutaneously or intramuscularly: in which event, absorption of the material might be too slow to provide an adequate stimulus for the vomiting reaction. Hammon (62) has suggested that kittens subjected to repeated intraperitoneal injections develop a low-grade peritonitis, which interferes with absorption of toxic materials and gives rise to an apparent tolerance; but such an explanation would hardly cover the resistance occasionally shown by previously uninoculated kittens. Davison, Dack, and Cary (63) reported that known enterotoxic filtrates, whose alpha-toxin had been neutralized by antiserum from appropriately immunized monkeys, evoked a higher incidence of vomiting reactions in kittens when given by the intracardiac rather than the intraperitoneal route. Hammon (62) injected filtrates boiled for 30 minutes into the saphenous vein of adult cats, and found the intravenous route provided a more specific and sensitive test for enterotoxin than did the intraperitoneal route.

Recently, in experiments of somewhat limited scope, we have verified Hammon's contention respecting the greater sensitivity of the test when the intravenous route is used. A few examples illustrating this point are shown in Table IV. Three large cats, each weighing about 2 kg., were given intravenously 5, 3 and 2 cc. respectively of the enterotoxic filtrate 12069 alpha (neutralized with Wood serum) previously described, and used in the experiments summarized in Table III. All the cats gave typical vomiting reactions, the severity of which was roughly proportional to the dose administered. The cat receiving 5 cc.

TABLE IV

COMPARISON OF SENSITIVITY OF FELINE REACTIONS TO ENTEROTOXIN INJECTED BY INTRAVENOUS AND INTRAPERITONEAL ROUTES

Animal Designation	Weight (gms.)	Vol. of filtrate and route of administration	Reaction
Black	2000	5 cc. intravenously	Vomited at 26′, 35′, 38′, 1¾ hrs; diarrhoea at 2½ hrs.; died in about 12 hrs.
Tawny	2220	3 cc. intravenously	Vomited at 42′, 2 hrs., 2¼ hrs.; diarrhoea at 1½ hrs.
White	2200	2 cc. intravenously	Vomited at 38′, 1½ hrs.; diarrhoea at 46′, 1½ hrs.
590	1550	4 cc. intraperitoneally	Nil
708	455	3 cc. intraperitoneally	Vomited at 44′, 54′.
709	455	3 cc. intraperitoneally	Nil
White paws	635	0.5 cc. intravenously	Vomited at 1¼ hrs.
Grey paws	630	1 cc. intraperitoneally	Nil

The same preparation of 12069 alpha filtrate, neutralized with Wood serum, was used throughout.

collapsed 1 hour after the injection, and died about 12 hours later, no particular abnormality being noted at autopsy. By contrast, a young adult cat of lesser weight failed to react to 4 cc. given intraperitoneally, while a kitten weighing 455 gm. also reacted negatively to 3 cc. by the same route. Another kitten of the same weight, however, reacted positively when similarly treated; while further positive reactions following intraperitoneal administration of 1, 2 and 3 cc. to kittens are exemplified in Table III. Again, of two kittens of the same weight (litter mates) one received 0.5 cc. of enterotoxin intravenously, and the other 1 cc. intraperitoneally. The former had a definite, though mild reaction, vomiting once only; but the latter, though listless, failed to vomit.

Although our experience with the intravenous route has not been extensive, it would seem to have the advantage over the intraperitoneal route of more consistent sensitivity. The main disadvantage of the intravenous route lies in the struggles sometimes involved in giving the injection to a refractory animal. Whether this route renders the test also more specific is dubious. The vomiting reaction ascribed to the beta-toxin occurs after intravenous, as well as after intraperitoneal injection; while, as will appear in the next section of this paper, feline vomiting reactions may be provoked by filtrates of some strains of *Proteus vulgaris* and *B. coli,* administered by either of these routes.

Several workers have alluded to the occurrence of non-specific kitten reactions, but when precautions were taken to avoid trauma to the intestines, they were not wittingly encountered in these laboratories. Admittedly, Woolpert and Dack (60) had referred to a strain whose filtrate caused profuse vomiting and diarrhoea in cats, although no enterotoxin could be detected by human and monkey feeding experiments; but this discrepancy was considered due to their filtrates having been injected without prior elimination of the alpha-toxin component. Again, boiled filtrates prepared from our strain 24 had given positive kitten reactions, despite rather large amounts of its untreated filtrates having been previously taken with impunity by human volunteers; but this was interpreted as evidence of high sensitivity, rather than of occasional non-specificity in the kitten test. Indeed, until human feeding and feline injection experiments were undertaken in parallel, it could hardly have been suspected that a syndrome so akin to staphylococcal food poisoning in man could be evoked in cats or kittens by two distinct toxins elaborated by the same species of micro-organism.

At the time of their first report on the kitten test, Dolman, Wilson and Cockcroft (51) had used filtrates which were either incubated with formalin, or kept in a boiling water bath for 30 minutes, until rabbit and sheep cell haemolysins were no longer detectable, or remained in only negligible amounts. Under these circumstances, vomiting reactions due to beta-toxin could rarely have been encountered, unless the haemolytic properties of the beta-toxin were destroyed more rapidly than its capacity to produce vomiting in kittens. But in later publications, Dolman and Wilson (59, 64) gave 15 or 20 minutes as the minimum time of exposure to 100°C. which generally sufficed to reduce the alpha- and beta-toxins to "insignificant" titres. In the light of present evidence, it seems probable that enough residual beta-toxin may be present to cause false kitten reactions when some filtrates are exposed to 100°C. for lesser periods than 30

minutes; and even exposure for 30 minutes does not necessarily preclude the occasional false reaction from a particularly heat-stable beta-toxic filtrate. Herein may be the explanation for the growing misconception that beta- and enterotoxin were identical; for the high percentages of supposedly enterotoxigenic strains among staphylococci isolated from various sources, and especially from raw milk; and for the discrepancy between such findings and the early human feeding experiments reported by Dolman (50), who concluded therefrom that food poisoning strains might not be widely distributed.

Suggested Modifications in the Kitten Test

The method of boiling filtrates prior to performance of the kitten test has hitherto commended itself on the grounds of simplicity and rapidity. Removal of the alpha- and beta-toxins through formalinization obviously entailed a delay of at least several days; while neutralization of these toxins by means of an appropriate antitoxic serum involved a relatively complicated procedure. However, it now seems clear that only by adopting one or other of these two methods is it possible to avoid the contingency that in order to destroy all the beta-toxin in a filtrate, it may have to be exposed to 100°C. long enough to reduce the enterotoxin below ordinarily detectable limits.* If all staphylococcus strains behaved like 12069 in showing an incompatibility between beta- and enterotoxigenic powers, the situation would be far simpler; for no beta-toxigenic strain could then be suspected of food poisoning propensity. But strains are known (e.g. Barss) which produce highly potent beta-toxin in addition to the alpha-toxin and enterotoxin.

Of the two alternative methods available for removing the alpha- and beta-toxins, the lapse of time required for detoxication by formaldehyde is a serious disadvantage, whereas the maintenance of a supply of an alpha+beta antitoxic serum of known neutralizing capacity should not be too troublesome. Experimental evidence to date suggests that the most specific and sensitive method of determining the enterotoxic content of a filtrate (apart from direct resort to human volunteers) is the intravenous administration of 1-3 cc., appropriately neutralized with alpha- and beta-antitoxin, to at least 2 kittens or cats. Unfortunately, use of such toxin-antitoxin mixtures is apt to induce serum sensitivity, rendering animals unsuitable for subsequent tests with filtrates containing serum. When a laboratory finds the neutralization method not feasible, filtrates boiled for 30 minutes may be given intravenously, with reasonable confidence in the specificity of the results obtained, especially when the strain in question is not beta-toxigenic. But it must then be borne in mind that although the majority of food poisoning strains may, in the laboratory, produce enterotoxin of sufficient potency and heat stability to give positive cat or kitten reactions, a negative reaction under such conditions to even a dosage of 5-6 cc. would not necessarily indicate absence of enterotoxin from the filtrate before boiling.

These modifications in the kitten test are proposed only tentatively, and chiefly as a basis for further study by others. Analysis of an intrinsically com-

**Whether or not such a contingency arises in a given instance will of course depend upon the initial potencies, and the degree of heat stability of these two components in the filtrate.*

plicated situation has not been made easier by the appalling difficulties encountered in securing an adequate supply of kittens or cats. By bribery or guile, after travelling far and exhausting much patience, over 200 kittens and cats were mustered for the experiments on which these recommendations are based. But numerous animals died of immaturity or infection before they could be used; and the seasonal breeding tendencies of cats have made it impossible during the winter months to investigate more intensively the numerous points which remain for confirmation and clarification.

Fresh surveys of the incidence of food poisoning strains of staphylococci, undertaken with due precautions to employ an animal test of high specificity and sensitivity, might possibly reveal some degree of selective distribution of these strains. It appears indeed that the main application of the kitten test is to determine such points as this, rather than to verify the enterotoxigenic power of strains implicated in food poisoning outbreaks. Dolman (42) has pointed out the fallacies underlying attempts to prove, for legal or other purposes, whether or not a given strain was responsible for some outbreak. Such attempts are, in any event, of little prophylactic value. To whatever extent new kitten test surveys should point to a restricted distribution of enterotoxigenic strains, the control of staphylococcal food poisoning hazards would be correspondingly simplified. But epidemiological and laboratory evidence suggests that such surveys would rather reveal a wide distribution, though a comparatively low incidence, of potential food poisoning strains. If this should prove the outcome, then control of staphylococcal food poisoning must still depend, as was pointed out in the beginning of this section, upon more enlightened efforts to reduce to a minimum the various opportunities for staphylococci to contaminate foodstuffs, and to multiply therein.

OTHER REPORTED TYPES OF BACTERIAL FOOD POISONING

In the public mind, the conception of food poisoning is still coloured by the entirely out-moded "ptomaine" hypothesis. Nearly a quarter of a century ago, Savage (65) showed that foodstuffs far too putrid to be consumed by human beings could be fed in enormous amounts to kittens without resulting harmful effects. Most of the foods were taken from blown tins of canned meat or fish, and Proteus organisms were chiefly responsible for their putrefaction. Savage concluded that the "action of putrefactive bacteria and their products is a minor, if not a negligible, matter in the production of toxic symptoms in man"; but he emphasized that decomposed food should always be condemned, on the grounds that it had obviously been exposed to contamination with bacteria, among which might be included those especially associated with food poisoning.

Previously, Savage (15) had critically examined and rejected the reports in the early literature of food poisoning outbreaks ascribed to *Proteus vulgaris* or *B. coli*. But the belief died hard that some strains of these organisms might occasion food poisoning. Jansen and den Dooren de Jong (66), for instance, reported the isolation of large numbers of *B. coli communior* from potato purée incriminated in a dramatic outbreak involving several hundred persons in Rot-

terdam in 1929. Further reference to this report will be made later. Jordan and Burrows (31) briefly summarized the more recent literature on food poisoning outbreaks ascribed to *Proteus vulgaris* and to *B. coli*, as well as to other miscellaneous bacteria. They claimed that certain strains of these organisms, after several transfers on a medium containing 1 per cent starch, yielded filtrates which caused monkeys to vomit when fed therewith. Filtrates prepared from such specially treated strains were apparently not administered to human beings. Moreover, the same authors state that ordinary broth cultures of several Proteus strains had been repeatedly fed to volunteers without deleterious effect; while Hunter and Dack (67) altogether failed to reproduce the vomiting reaction in monkeys with filtrates from strains of a variety of organisms, including *B. coli*, Proteus, and certain Salmonella types, which had all been repeatedly transferred on the starch-containing medium described by Jordan and Burrows. On the other hand, Minett (38) obtained kitten vomiting reactions from intraperitoneal injection of filtrates from 4 strains of *B. coli* isolated many months previously from calves with "white scours", and from a cow with acute mastitis. Finally, Wilson and Fulton (58) encountered numerous food poisoning outbreaks of "toxin" type in war-time England, in which the bacteriological evidence was inconclusive. *B. coli, Proteus vulgaris,* or Gram-positive sporulating bacilli proved not infrequently to be the predominant organisms isolated from the peccant foods, with staphylococci and streptococci sometimes also present. The fact that filtrates prepared from certain of these organisms had caused vomiting reactions when injected intraperitoneally into kittens, seemed to Prof. Wilson to warrant further investigation of their enterotoxigenic capacity; and a representative group of such strains was forwarded to these laboratories with this end in view.

Streptococci, mostly of green-producing type, have in recent years been reported as the predominant organisms in foodstuffs implicated in several outbreaks of food poisoning. Linden, Turner and Thom (68), for instance, reported two outbreaks due to cheese from which very large numbers of *Streptococcus lactis* were isolated. Milk cultures of these organisms fed to dogs and cats provoked diarrhoea, commencing a few hours later and lasting for several days. Cary, Dack and Meyers (69) isolated a green-producing streptococcus from canned sausages which had caused nausea, vomiting, colic and diarrhoea in 75 boys. Portions of similarly-infected sausages from previously unopened cans were eaten by a volunteer, who felt ill 5 hours later, and for 24 hours thereafter had nausea interspersed with two periods of vomiting and epigastric pain. This was followed by marked exhaustion and constipation. Similar symptoms occurred in another volunteer who drank 40 cc. of a filtrate containing some living streptococci; whereas no ill effects were suffered by a third volunteer who drank 50 cc. of bacteria-free filtrate. Mice, guinea pigs and monkeys were fed living cultures without apparent effect, although kittens given 48-hour milk cultures had soft stools the next day. Jordan and Burrows (70) also isolated a pure culture of *Streptococcus faecalis* from the filling of a cocoanut cream pie, portions of which had apparently caused some cases of food poisoning. Sterile culture filtrates prepared from this strain produced

vomiting and lassitude a few hours after being fed to monkeys in 25 cc. amounts. Of 4 strains of green-producing streptococci isolated from human infections by these authors, one yielded a filtrate which gave positive reactions in monkeys, while the remaining 3 strains proved negative in this respect. Moreover, 2 of 6 freshly-isolated strains of *Streptococcus haemolyticus,* all of human origin, yielded filtrates of which 25 cc. amounts caused vomiting in monkeys. Minett (38) obtained negative kitten reactions on injecting intraperitoneally the boiled filtrates of 15 strains of beta-haemolytic *Streptococcus agalactiae* isolated from the milk of cows with mastitis. But when unheated filtrates were used, 2 among 6 strains tested gave positive results.

Finally, Cary, Dack and Davison (71) recorded an outbreak involving 117 persons, apparently due to beef croquettes heavily infected with green-producing streptococci. Abdominal cramps and diarrhoea were the main symptoms, nausea and vomiting affecting only a small percentage of the cases. The incubation period ranged from 6 - 18 hours, averaging 12 hours; and the symptoms subsided in 6 - 24 hours from onset, except in 4 cases, where they persisted for over 3 days. Of 5 volunteers who took 20 cc. of filtrate prepared from this strain, none suffered any ill effect; but when living broth cultures were swallowed, 5 out of 7 volunteers experienced cramps and diarrhoea commencing about 12 hours later.

Against this background of conflicting reports in the literature, an extensive series of feeding experiments was planned, using human volunteers. Interest centred first on the cultures which had been isolated in England from incriminated foodstuffs; and upon the report of Wilson and Fulton (58) that filtrates prepared from certain of these strains had given positive kitten reactions.

False Positive Kitten Reactions from Proteus and B. coli Filtrates

Filtrates were prepared according to the soft agar-partial CO_2 atmosphere method from *Proteus vulgaris,* strain 16122, and from *B. coli,* strain 14061 C. The Proteus culture had been isolated, along with large numbers of Anchromobacterium and Enterococcus, from pressed beef which had apparently caused diarrhoea in 25 persons about 10 hours after its consumption. The *B. coli* culture had been isolated, along with large numbers of *Streptococcus viridans* and *Streptococcus faecalis,* from meat and vegetable pie which was the alleged cause of minor abdominal symptoms in some A.R.P. workers. Filtrates from both strains had given positive kitten reactions in England, and our own observations accord with these findings. Table V, in which representative results obtained with these filtrates are set forth, shows that the kitten vomiting factor elaborated by the Proteus culture was destroyed by boiling for 30 minutes, while the *B. coli* filtrate contained a heat-stable kitten vomiting factor.

Two large cats injected intravenously with 1 and 3 cc. respectively of filtrate 14061 C also gave vomiting reactions, although no diarrhoea occurred; while 3 cc. of filtrate 16122 evoked several bouts of vomiting in another large cat when given intravenously. These feline reactions were very similar to those produced by staphylococcus enterotoxin, although perhaps more lassitude and malaise were associated with the latter; and all the animals appeared quite well

next morning. Unheated filtrates from two other Proteus strains, from two strains of *B. coli,* and from two strains of aerobic spore-bearing bacilli, likewise isolated in England from suspected foodstuffs, failed to cause vomiting on intraperitoneal injection into kittens, although one kitten collapsed and died overnight following injection of 3 cc. of filtrate prepared from a Gram-positive sporulating bacillus.

Whether the kitten-vomiting factors produced by these strains of *B. coli* and of *Proteus vulgaris* are antigenic has not yet been determined; but for the present, the important point is that the reactions they evoked, like those produced by staphylococcus beta-toxin, appear to be without significance from the standpoint of food poisoning potentiality.

TABLE V

KITTEN REACTIONS TO INTRAPERITONEAL INJECTIONS OF BACTERIAL FILTRATES

Proteus vulgaris 16122

Kitten No.	Weight (gms.)	Volume injected	Reaction
		cc.	
593	575	2	Restless, shivering, 50′—1½ hrs. later.
558	470	3	Vomited 34′, 39′, 40′.
592	470	3	Vomited 45′, 50′, 54′, 2 hrs.; diarrhoea 46′, 51′, 56′, 62′, 1 hr. 35′, 2 hrs.
591	585	4 (boiled)	Loose stool 70′.

B. coli 14061 C

Kitten No.	Weight (gms.)	Volume injected	Reaction
		cc.	
696	535	2	Vomited 53′, 62′; diarrhoea 33′, 36′.
699	620	2	Vomited 56′; diarrhoea 21′, 63′.
595	335	3	Vomited 33′, 37′, 38′, 43′, 45′; diarrhoea 40′
697	680	3	Vomited 37′; diarrhoea 10′, 1¾ hrs.
700	660	3	Vomited 2 hrs.; diarrhoea 19′, 55′, 2 hrs.
695	700	4 (boiled)	Vomited 2 hrs., 2¼ hrs.; diarrhoea 19′.
698	775	4 (boiled)	Vomited 2 hrs. 10′; diarrhoea 15′, 1¾ hrs.
596	935	5 Broth	Nil

Ingestion of Filtrates and Cultures of Various Organisms by Humans

Bacteria-free filtrates were prepared from 3 strains of *Proteus vulgaris,* 2 strains of *B. coli,* 2 strains of spore-bearing Gram-positive bacilli, and from 2 strains of *Streptococcus viridans,* all of which were grown by the soft agar-partial CO_2 atmosphere method. Amounts of these filtrates ranging from 2 - 50 cc. were then taken in various vehicles by laboratory colleagues and students. Table VI shows the foodstuffs from which the various cultures were first isolated, the number of organisms reported present per gram of the suspected foods, and the volumes of filtrates taken by the volunteers. The final column shows the weights of the original foodstuffs which, by calculation, would have contained approximately the same numbers of organisms as were present, before filtration, in the different volumes ingested. Although the amounts thus taken of the soluble metabolites of the various cultures were far larger than would have been consumed in a helping of the original foodstuff, there was no

evidence whatever of the presence in the filtrates of substances enterotoxic for man. The only effect noted was an increasing antipathy to this form of nourishment, due rather to the peculiar foulness of the odour and flavour of many of the filtrates (which no amount of ingenuity seemed able totally to mask) than to fear of a positive reaction.

TABLE VI

INGESTION OF FILTRATES FROM ORGANISMS ISOLATED FROM SUSPECTED FOODSTUFFS

Strain Designation, and Origin	Numbers Originally Present Per Gram	CC. of Filtrate Ingested by Different Volunteers	Equivalent Weight of Original Foodstuff
Proteus vulgaris 16122 (Pressed Beef)	2.4×10^8	10, 15, 30, 40, 50	400-2000 gm.
15902 (Pressed beef)	6×10^6	5, 5, 5, 10, 10, 10, 10	10-20 kg.
15221 (Pressed beef)	2.5×10^8	5, 5, 5, 5, 10	20-40 kg.
B. coli 14061 C (Meat and vegetable pie)	3.3×10^5	10, 10, 30, 30, 30, 40, 50	300-1500 kg.
12569 (Raw sausages)	7×10^6	5, 5, 10	10-20 kg.
Aerobic spore-bearing Gram-positive bacillus 14061 D (Meat and vegetable pie)	4.4×10^4	5, 5, 5, 10	1000-2000 kg.
11136 (Canned peas and carrots)	2.5×10^7	2, 2, 2, 2, 2, 4, 4	1-2 kg.
Streptococcus viridans B. (Rissoles)	2.5×10^8	5, 5, 10, 10, 20, 20	200-800 gm.
C. (Rissoles)	5×10^8	5, 5, 10, 10, 20, 20	100-400 gm.

Several culture filtrates were tested in addition to those summarized in Table VI. For instance, from Dr. Forrest Fulton were received 2 strains of green-producing streptococci, isolated from rissoles; and also one strain of a haemolytic coliform bacillus, and one strain of a haemolytic enterococcus, isolated from cheese. Filtrates prepared from each of the 2 streptococcus strains were taken by different volunteers in the following amounts: 5, 5, 10, 10, 20 and 20 cc. Two volunteers took 5 and 10 cc. respectively of the haemolytic coliform filtrate; while two others took 2 and 10 cc. of the haemolytic enterotococcus filtrate. In no instance was there any reaction.

The foregoing experiments were all carried out within a few months of the isolation of the cultures. But Jordan and Burrows (31) had claimed that cultures might lose their enterotoxigenic capacity (as judged by monkey vomiting reactions) under laboratory conditions; and despite their report having remained unsubstantiated, it seemed desirable to test filtrates from a few freshly isolated strains on human beings. Accordingly, culture filtrates were prepared

from 3 Proteus strains isolated from human faeces a few days previously in the Provincial Laboratories. Twelve persons took amounts ranging from 5 - 20 cc. of these filtrates, without ill effect. Culture filtrates were also prepared from 4 recently isolated strains of *Streptococcus haemolyticus* (1 per cent beef serum being added to the usual medium to promote growth), and were taken by mouth by different persons in the amounts indicated:

Culture McComb C. Group C streptococcus, isolated from raw milk which had allegedly given rise to numerous gastro-intestinal disturbances:
3, 3, 10, 10, 10, 10, and 10 cc.

Culture McComb A. Group A streptococcus, isolated from another sample of milk from the same herd as above:
25, 25, and 30 cc.

Culture 437. Group A streptococcus, isolated from human throat:
10, 25, 25, and 25 cc.

Culture 4376. Group A streptococcus, isolated from human throat:
10, 10, 25, and 25 cc.

Again, no significant reactions occurred.

The possibility was recognized that non-filtrable enterotoxic factors, which would not be detected in experiments of the foregoing type, might under some circumstances be manufactured by certain organisms in appropriate foodstuffs. An attempt was made to cover this contingency by giving volunteers meat pies inoculated with cultures of certain organisms which had been already tested by the methods described above. Small meat pies, each weighing 85 - 130 gm., were inoculated through the crust with 1 cc. amounts of an overnight broth culture of the organism under test. The pies were then incubated for about 8 hours at 37°C., when volunteers each consumed one pie. Bacterial counts were made of the suspension, by the poured plate method, so that the numbers of organisms inoculated were known; while similar counts made on small samples of the meat removed from the pies just prior to their consumption, permitted the numbers of organisms then present per gram of meat to be calculated. From Table VII it is evident that a considerable degree of bacterial multiplication took place within the pies during the 8 hours of incubation. Further, their final bacterial contents (even after making allowances for doubtless lesser counts in the pie crust) were so much higher than those originally reported for the implicated foodstuffs, that the numbers of organisms consumed by each volunteer must have greatly exceeded the numbers taken unwittingly by the victims in the various food poisoning episodes. The flavour of the pies was not apparently altered by this treatment, and no ill effects followed their consumption.

There are obviously abundant opportunities for contamination of certain foodstuffs with *B. coli, Proteus vulgaris,* and the other organisms previously mentioned; and once pollution has occurred, such organisms will multiply in the food to an extent limited only by its suitability as a culture medium, by the nature and numbers of other organisms present, and by the time and temperature of incubation prior to consumption. Before reaching the laboratory for examination, samples of the suspected foodstuff have usually been exposed to further contamination and to delays in transit. Under such circumstances, it seems inevitable that very large numbers of air- or finger-borne organisms should at times be found

in foods. A reconsideration of the literature reveals that food poisoning potentialities have been attributed to these organisms chiefly on the basis of their occasional predominance in suspected foods. But the foregoing experiments on human beings show how fallacious this argument may be. Moreover, the animal experimental evidence adduced in support of a food poisoning role for such organisms now seems quite unconvincing. The report of Jansen and den Dooren de Jong (66) that mice developed diarrhoea when given bread soaked in a broth culture of the *B. coli* strain allegedly responsible for a typical "toxin" outbreak

TABLE VII

CONSUMPTION OF MEAT PIES ARTIFICIALLY INFECTED WITH CULTURES SUSPECTED OF CAUSING FOOD POISONING

Culture Designation, and Origin	Numbers Originally Present per Gram	Numbers Introduced into Meat Pie	Numbers Finally Present per Gram of Meat
Proteus vulgaris 16122 (Pressed beef)	2.4×10^8	3.7×10^8	Pie 1. 4.6×10^9 Pie 2. 3.2×10^9 Pie 3. 5.3×10^9 Pie 4. 1.5×10^9
B. coli 14061 C (Meat and vegetable pie)	3.3×10^8	2.8×10^9	Pie 1. 2.9×10^9 Pie 2. 4.65×10^9 Pie 3. 1.5×10^9 Pie 4. 3.4×10^9
Streptococcus viridans C. (Rissoles)	5.25×10^8	2.1×10^8	Pie 1. 3.9×10^9 Pie 2. 2.75×10^9 Pie 3. 2.8×10^9 Pie 4. 1.8×10^9
Aerobic spore-bearing Gram-positive bacillus C. 11136 (Canned peas and carrots)	2.5×10^7	3.4×10^7	Pie 1. 2.45×10^8 Pie 2. 1.35×10^8 Pie 3. 3.3×10^8 Pie 4. 1.7×10^8

Meat pies weighed 85-130 gms. each.
One pie consumed by each volunteer.

of food poisoning, is rendered of no special significance by the authors' own admission that similar effects were produced by other strains of *B. coli* having no relationship to food poisoning.* Again, the claim of Jordan and Burrows (31) that filtrates which proved enterotoxic for monkeys could be prepared from certain strains of Proteus, Salmonella, *B. coli,* and several other species, has not been confirmed: in fact, Hunter and Dack (67) reported entirely contrary results. Further, even if these vomiting reactions had been reproduced in monkeys by other workers, their reliability as indicators of substances enterotoxic for man would still need verification. Finally, the kitten vomiting reactions obtained by Minett (38) with certain *B. coli* filtrates, and by Wilson and Fulton (58) with filtrates of some strains of *B. coli* and *Proteus vulgaris,* have been shown to be unrelated to the presence of food poisoning factors. In sum, although a much larger number of feeding experiments would need to be carried out on humans,

*The fact that several workers have reported toxic effects from injection of B. coli filtrates into laboratory animals is here no more relevant than in the case of the Salmonella group, to the question whether such organisms might on occasion assume a food poisoning role.

using many strains freshly isolated from suspected foodstuffs, before the categorical statement could be justifiably made that organisms of the species in question play no part in bacterial food poisoning, there is at present no significant evidence pointing to such a role.

The contention that occasional strains of streptococcus may be capable of causing a relatively mild food poisoning syndrome, finds somewhat more support. Our own series of experiments with streptococcal filtrates are in accord with those of Cary, Dack and Meyers (69), and of Cary, Dack and Davison (71), who failed to note ill effects in volunteers given filtrates of 2 strains of green-producing streptococci isolated from incriminated foods. But live cultures of these strains provoked abdominal distress and diarrhoea, with general malaise, in volunteers, the symptoms being similar in character to those of the victims in the original outbreaks. These findings, and the fact that the average incubation period was 12 hours, lend validity to the contention of Cary, Dack and Davison that the postulated streptococcal food poisoning syndrome is due to infection with living organisms, and not to preformed toxic metabolites.

Despite Jordan and Burrows' reference to monkey vomiting reactions following the feeding of *Streptococcus haemolyticus* filtrates, food poisoning propensities have not been ascribed to these organisms. Yet nausea and vomiting, and sometimes diarrhoea, occur in scarlet fever; and milk-borne outbreaks of this disease, and of epidemic sore throat, represent undoubted instances of food-borne infection. Moreover, since vomiting may result from the injection of excessive amounts of scarlet fever toxin, it is conceivable that foodstuffs might occasionally provide the conditions necessary for elaboration of a sufficient quantity of streptococcus toxin to induce vomiting in the consumer. But such occurrences must be very rare; for although Dick and Dick (72) allude to vomiting induced by oral administration of purified toxin in powder form, they administered to children without harmful effect several successive doses of undiluted toxin containing 40,000 skin test doses per cc., in amounts up to 30 cc.

Enterotoxigenic Staphylococci Overgrown by Other Bacteria in Foodstuffs

If enterotoxic properties cannot be ascribed to the various organisms discussed in the preceding section, some alternative explanation must be sought for those outbreaks in which they feature as the predominant bacteria isolated from the implicated foods. The hypothesis is advanced that many such outbreaks are actually due to staphylococcal food poisoning. It is in keeping with this hypothesis that the symptomatology in such outbreaks should point characteristically to preformed toxin, rather than to infection, as the responsible factor. Successive Ministry of Health Reports, as well as Savage (8), Wilson and Fulton (58), and many others, have bewailed the lack of a verifiable aetiological agent in the high percentage of "toxin" type outbreaks of food poisoning now customarily reported as of unknown origin. Yet staphylococcus enterotoxin is the only agent which has been proved beyond question capable of simulating the "toxin" type of food poisoning in man. Moreover, the foodstuffs implicated in these outbreaks are similar to those commonly involved in staphylococcal food poisoning. Furthermore, staphylococci are often present, even though sometimes in relatively

small numbers, in the peccant food in episodes of this type. Such lesser numbers of staphylococci have usually been looked upon as fortuitous, and of no special significance; but they may on occasion rather represent the survivors of far larger numbers initially present, as the following arguments and experimental findings indicate.

Public health laboratory workers who frequently conduct bacteriological examinations of foodstuffs involved in outbreaks of "toxin" type, are familiar with the fact that even when staphylococci are present in almost pure culture, their numbers per gram of suspected material may lie within a very wide range. Occasionally, the count may be very high indeed, as in the following instance:

Several persons ate portions of a recently cooked ham without ill effect. The ham was kept outside the house in the shade, in spring weather. One person partook of the ham 29 hours later, and became ill with a typical staphylococcal food poisoning syndrome. Next day, 3 other persons took some of the ham, and likewise became ill. When examined within 24 hours at the Provincial Laboratories, a portion of ham taken from just beneath the surface gave a count of 200 million staphylococci per gram, about one half of the colonies being pigmented and haemolytic. A sample taken about 6 cms. below the surface gave a count of 175,000 staphylococci per gram.

Counts of from 100,000 to one million staphylococci per gram of material are more usual. Such low counts can only be reconciled with enterotoxic properties in a given food by supposing that a marked decline in bacterial population occurs after an early phase of rapid proliferation with maximal enterotoxin production. This decline could result from exhaustion of the nutritive value of the foodstuff, and would usually be accelerated by competition with another organism. When a rampant Proteus strain be the competing organism, it may prove impossible to detect any staphylococci in the foodstuff by the ordinary methods of culture on blood plates. Alternatively, although plates receiving a heavy inoculum of a suspension of the foodstuff may be so overgrown with Proteus that no staphylococcus colonies are detectable, if a high dilution of the suspension is used, staphylococci may sufficiently outnumber the Proteus to grow without hindrance. Instances of such situations have been encountered in the Provincial Laboratories (involving chicken gravy and ham respectively), wherein the symptomatology of each outbreak was typical of staphylococcal food poisoning. A third possibility, liable to be manifested in foodstuffs of long keeping qualities, is that no staphylococcus colonies, and only a few colonies of Proteus or other organisms, may be isolated. The following account perhaps illustrates this contingency.

A man and his wife and 2 children ate at 3 p.m. some chocolates just purchased in a department store. Two hours later, all 4 persons were taken ill with vomiting. The father, who had eaten more chocolates than the others, also had diarrhoea, and all his symptoms were more severe. Another child came home from school at 5.30 p.m., allegedly ate one chocolate only, and was taken similarly ill at 7.30 p.m. All had recovered by next day. An emulsion of the chocolates plated out in the Provincial Laboratories yielded a few colonies of a Gram-positive bacillus and a slightly larger number of Proteus colonies. No staphylococci were found.

A sample of the melted chocolate was inoculated with staphylococci, and kept at 37°C. At the end of 24 hours, the bacterial count showed a marked

increase. After 3 days, the count had fallen considerably, and at 5 weeks viable staphylococci were no longer detectable. While this experiment merely indicates that a strain of staphylococcus can multiply briefly, and remain viable for several days in chocolate, the findings are not inconsistent with the suggestion that the episode described above was due to staphylococcus enterotoxin remaining in the chocolates long after the organisms which produced it were extinct. The presence of the Proteus colonies can be ascribed to the handling which chocolates undergo, especially during the dipping process; and to the custom of keeping the vats of melted chocolate at about 32°C. for long periods. A reformed sanitation of chocolate manufacture is apparently overdue.

The following experiment was carried out in a final effort to substantiate the hypothesis that enterotoxigenic staphylococci in a foodstuff may be masked by a subsequent formidable overgrowth of some other organism, innocent of any food poisoning role.

Four sausages (wieners) each weighing 40-50 gm. were boiled, and samples shown to be bacteriologically sterile. The sausages were then inoculated with an overnight broth culture of the enterotoxic Staphylococcus 12069 alpha. Each wiener was injected with a few drops of the culture at its ends and middle, so that a total inoculum of about 0.5 cc. was made, representing roughly 150 million viable staphylococci according to plate counts. The wieners were then kept under aseptic conditions at room temperature (22°C.) in individual jars. After 24 hours, plate counts were made on each wiener, using dilutions of emulsions prepared from sample portions. The counts ranged from 100 million to 2,000 million staphylococci per gm.

A further inoculum of 0.5 cc. of an overnight broth culture of *Proteus vulgaris* 16122, representing roughly 200 million viable organisms according to plate counts, was then injected into each wiener, and incubation at 22°C. resumed under aseptic conditions for another 28 hours. At the end of this period, the wieners smelt somewhat stale and pungent, and kittens refused to eat portions of one offered to them. However, two persons each selected at random one of the remaining wieners, and ate about one quarter thereof, finding the taste not significantly abnormal. A dog weighing about 15 kg. ambled into the laboratory at this juncture, appearing hungry, and was given portions of two sausages. One human subject (C.L.A.) felt nausea after 1½ hours, followed ½ hour later by the first of 6 bouts of vomiting, accompanied by severe diarrhoea; and next day, she was still feeling unwell. The other subject (C.E.D.) had slight cramps, eructations and increased salivation, but no vomiting occurred. The dog became very quiet and vomited after 5½ hours. Plate counts made on samples from each wiener at the time of consumption showed that the staphylococci had undergone a great increase in numbers, the different sausages showing a range of from 3,000 million to 14,000 million organisms per gram. No Proteus colonies were noted.

Four days after the sausages had been inoculated with staphylococci, and 3 days after the Proteus culture was introduced, plate counts were again made on samples from the remaining portions of the sausages. Only Proteus colonies were now noted on the plates, and the counts ranged from 4,000 million to 50,000

million per gram of the different samples. Counts were made 3 days later (one week after the staphylococcus, and 6 days after the Proteus inoculations). Proteus counts were now very similar in all 4 samples, ranging from 30,000-50,000 million organisms per gram. Some staphylococcus colonies were also present, but they were so greatly outnumbered by the Proteus colonies that a casual scrutiny of the plates would have overlooked them. All counts were estimated by the poured plate method, using 1 cc. amounts of sausage suspension diluted to 1 : 10 million, 1 : 100 million and 1 : 1000 million.

By now, the remaining portions of sausage had an extremely unpleasant appearance and smell. Proteolytic breakdown was obviously occurring in patches, and there were some green spots. To eat them was deemed impossible, so they were pooled, macerated in saline, and the emulsion left overnight in the refrigerator. As the emulsion was still homogeneous next morning, further quantities of saline were added, and the material centrifuged. A fair degree of separation was thus achieved, and the slightly opalescent supernatant was pipetted off. To the 45 gm. of pooled, decomposing wiener, a total of 125 cc. of saline had been added, and 65 cc. of supernatant extract were harvested. Three volunteers, L.E.R., C.L.A., and C.E.D., each took 10 cc. of this extract, a dose which can be readily calculated to represent about 2.7 gm. of sausage. All 3 persons, the last two in particular, suffered very severe reactions. The results of this experiment are summarized in Table VIII.

TABLE VIII

COLONY COUNTS IN WIENER SAUSAGES INOCULATED WITH ENTEROTOXIGENIC STAPHYLOCOCCUS 12069 AND PROTEUS VULGARIS 16122

Approximate Colony Counts in Millions per gm. Wiener

Time	Wiener: 1	2	3	4
24 hours after inoculation of 150 million staphylococci	300	400	2,000	200
	(Only staphylococci seen in all plates)			
52 hours after inoculation of staphylococci: 28 hours after inoculation of 200 million Proteus	10,000*	3,000	13,000	14,000
	(Only staphylococci seen in all plates)			
4 days after inoculation of staphylococci: 3 days after inoculation of Proteus	40,000	50,000	30,000	4,000
	(Only Proteus colonies seen in all plates)			
7 days after inoculation of staphylococci: 6 days after inoculation of Proteus	30,000†	50,000	40,000	50,000
	300	500	1,500	400
	(Upper figures represent Proteus, lower figures staphylococcal, colonies)			

*About 10 gm. of Wiener 1 caused severe enterotoxic reaction in subject C.L.A.; 10 gm. of Wiener 4 caused slight reaction (no vomiting) in subject C.E.D.; 40 gm. of Wiener 3 + 10 gm. of Wiener 4 caused dog to vomit 5½ hours later.

†Balance of wieners pooled, emulsified, and the equivalent of 2.7 gm. each taken by subjects L.E.R., C.L.A., and C.E.D. All suffered severe enterotoxic reactions.

The Proteus culture used in this experiment was originally isolated from suspected food, and its filtrates had given kitten vomiting reactions. Although culture filtrates, and also meat pies containing the live culture, had failed to affect humans, it seemed desirable to exclude the possibility that any enterotoxic

factor was elaborated by this strain of Proteus, under the conditions of the sausage experiment. Accordingly, another group of wieners was inoculated, each sausage receiving 1 cc. of a broth culture containing 300 million *Proteus vulgaris* 16122 per cc. After 7 days, when putrescence had again set in, plate counts were made as before, and portions of the wieners were macerated in saline. The same 3 persons each took 15 cc. of the supernatant after centrifugation, this time without effect. The Proteus counts of the wieners at the time of their maceration were at least as high as those shown in the earlier experiments, while the dose of extract ingested was 50 per cent greater.

The reactions induced on the former occasion were thus undoubtedly due to staphylococcus enterotoxin, although bacteriological examination of the sausage at the time of consumption seemed rather to implicate *Proteus vulgaris*. Had the sausages been consumed 3 days earlier, similar reactions would almost certainly have occurred; yet at this time, no staphylococcal growth was apparent on the plates. Moreover, if the Proteus culture had been introduced into the sausage prior to the staphylococcus, it seems possible that effective amounts of enterotoxin might have been elaborated without conspicuous numbers of staphylococci being noted at any stage. Further, where the temperature of exposure of a doubly-infected foodstuff is higher than in the above experiment, the whole sequence of events may be greatly accelerated. In the outbreak described by Jansen and den Dooren de Jong (66) for example, whose symptomatology was absolutely typical of staphylococcal food poisoning, the suspected potato purée was kept near a hot stove for possibly 12-14 hours before being served at a banquet. This period is ample for the elaboration of enterotoxin under such conditions. By the time the laboratory examination was conducted several hours later, the staphylococci could quite conceivably have been outgrown by *B. coli*, to which responsibility for this outbreak was ascribed.

Again, in the light of the foregoing experiment, by postulating the coincident presence of staphylococcus enterotoxin and a living Salmonella strain in an incriminated food, our hypothesis can be stretched to explain those Salmonella outbreaks in which the occasional case manifests a brief incubation period, as in the report of Hinden (6). Although it is not justifiable as yet to go further, and contend that enterotoxigenic staphylococci are solely responsible for those attacks of food poisoning characterized by a short incubation period, by bouts of nauseau, vomiting and diarrhoea, and by quick and complete recovery, we cannot subscribe to the view expressed by Topley and Wilson (73) in the following quotation:

> "The pendulum is in fact slowly swinging in the direction of the old 'ptomaine' theory, with the distinction that the poisons now regarded as responsible are not the result of advanced protein decomposition, but are products of the growth in the food of those bacteria which are able to proliferate enormously without greatly altering the appearance and taste of the food. These products are most probably the autolyzed bacterial bodies themselves."

The findings recorded in this paper are believed to afford a not inconsiderable stimulus to the pendulum to reverse its direction again.

SUMMARY

1. An increased incidence of food poisoning is to be anticipated as a result of wartime conditions. Present sanitation standards are too low, and are now required to carry added burdens; while the public is apt to be less fastidious regarding food, and is generally ignorant or careless about the prophylaxis of air, droplet, finger and fly-borne contamination.

2. Human and animal Salmonella carriers are of higher incidence than is often supposed. The bounds of the Salmonella food infection hazard have also been extended by the recognition that a variety of Salmonella organisms formerly thought to be animal pathogens only, are potentially pathogenic for man.

3. A critical examination of the literature bearing on the Salmonella "toxin" hypothesis finds the experimental evidence inadequate to explain on this basis those food poisoning outbreaks which are characterized by brief incubation periods, explosive but transitory bouts of vomiting and diarrhoea, and early recovery.

4. Enterotoxigenic staphylococci account for the most prevalent type of bacterial food poisoning. Such strains of staphylococcus are probably of widespread distribution, but their actual incidence may be fairly low. The figures obtained hitherto in surveys based on the kitten vomiting reaction are likely to be too high, owing to the inclusion of false reactions due to residual beta-toxin in the filtrates under test.

5. Filtrates prepared from a beta-toxigenic variant of a food poisoning strain of staphylococcus received from England failed to cause gastro-intestinal disturbance in human volunteers, although the parent organism, which produced no beta-toxin, yielded filtrates of which 1 cc. caused severe vomiting and diarrhoea in several persons. Sixteen severe reactions in volunteer subjects are described. Three persons each underwent the experience 4 times over periods of 4-6 months, without developing any apparent immunity.

6. Staphylococcus beta-toxin may be sufficiently heat-stable to resist boiling for 20-30 minutes in such amounts as may evoke vomiting and diarrhoea when 1-4 cc. of filtrate are injected intraperitoneally into kittens. The incubation period of the reaction thus induced is usually less than when enterotoxin only is present in the filtrates. Death often occurs overnight following the injection of beta-toxin, whereas prompt recovery as a rule follows the true enterotoxin reaction.

7. Preliminary trial of intravenous administration of filtrates to kittens and cats confirms the greater sensitivity of the test when this route is used. Prior neutralization of the alpha- and beta-toxin components of filtrates with appropriate antisera is advocated, since any enterotoxin present may be brought below detectable levels if the alternative treatment by boiling is maintained long enough to eliminate false reactions through destruction of all beta-toxin.

8. Feline vomiting reactions, resembling those due to staphylococcus enterotoxin, were provoked by intraperitoneal and intravenous injection of filtrates from single strains of *B. coli* and *Proteus vulgaris*, isolated in England from incriminated foodstuffs.

9. Filtrates prepared from several strains of *Proteus vulgaris, B. coli,* Gram-positive sporulating bacilli, and streptococci, were taken by numerous persons in amounts up to 50 cc. without harmful effects. Meat pies inoculated with cultures of some of these organisms were also eaten without ill effects, although their bacterial counts greatly exceeded those found in the foodstuffs from which the cultures were first isolated.

10. The literature offers no evidence definitely implicating organisms of the foregoing species in the "toxin" type of food poisoning, although food heavily infected with some strains of green-producing streptococci may apparently cause relatively mild symptoms, the incubation period averaging 12 hours.

11. The hypothesis is advanced that the "toxin" type of food poisoning outbreak, so frequently reported as of unknown origin, is in reality usually due to enterogenic staphylococci which may be masked, overgrown or even extinct when the peccant food is examined in the laboratory. This hypothesis was strengthened by an experiment in which 3 persons were made violently ill by consuming a saline extract of 2.7 gm. of wiener sausage which had been inoculated with enterotoxigenic staphylococci, and subsequently with *Proteus vulgaris.* At the time of consumption, the wieners were decomposing, and the Proteus counts were 30,000-50,000 million organisms per gram, outnumbering the staphylococci a hundredfold. At a prior stage, when the wieners were already toxic, no staphylococci could be detected on poured plates. Larger amounts of extract from wieners equally infected with the same strain of *Proteus vulgaris,* but not inoculated with staphylococci, were eaten without ill effect by the same 3 volunteers.

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The Effect of Glucose on Penicillin Potency Tests*

A. G. LOCHHEAD AND M. TIMONIN

IN estimating the bacteriostatic potency of culture fluids of *Penicillium notatum*, using the serial dilution method of assay, it soon became apparent that the values obtained varied greatly depending upon the nutrient solution used for the test organism, *Staphylococcus aureus*. Reports by other workers using this method of assay indicate that various media have been and are being used. The pronounced differences we have noted between titres, particularly where glucose broth has been compared with plain nutrient broth, emphasize the impossibility of any accurate comparison of results when different procedures are followed.

For the purpose of the assay 1 ml. of the bacteria-free culture fluid of the fungus was transferred to a tube containing 9 ml. of the assay medium. From this 1-10 dilution a further series of dilutions was made in the same medium. Inoculation was made by transferring a 3-mm. loopful of a 24-hour culture of the test organism in nutrient broth and tubes were read after 24 hours' incubation at 37°C.

A few examples suffice to illustrate the effect of adding 1 per cent glucose to nutrient broth upon the assay values. Results for three strains of *P. notatum* grown in modified Czapek-Dox medium are given in table I, and show the widely divergent results which may be obtained in a determination of the potency of a crude penicillin solution.

TABLE I

INHIBITION OF *Staphylococcus aureus* (292) BY CRUDE CULTURE FLUIDS OF *Penicillium notatum*

P. notatum strain No.	Age of *Penicillium* culture (days)					
	5	10	15	20	25	30
	Highest inhibiting dilutions—nutrient broth					
437	40	80	40	40	20	<10
443	>320	160	40	<10	<10	<10
451	40	80	40	<10	<10	<10
	Highest inhibiting dilutions—glucose broth					
437	160	320	320	80	80	80
443	>320	>1600	1600	1600	1600	3200
451	160	1280	800	1600	1280	<10

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The effect of different sugars upon the assay values was studied in a number of tests in which glucose, sucrose, lactose and mannose were added respectively in 1 per cent concentration to a basal nutrient broth medium. Table II gives the titre values for a 20-day culture fluid of *P. notatum*, strain 437, for the testing of which three strains of staphylococci were used. In all cases the addition of glucose results in greatly increased bacteriostasis for all strains of staphylococci. In the presence of sucrose the titre is higher than that of the nutrient broth controls, though still considerably lower than the values with glucose. The addition of lactose and mannose tested in this and other series resulted in no change as compared with the controls. The results in table II indicate that, in addition to the test medium, the strain of organism used is a factor in determining the titre and that different staphylococci vary in their resistance.

TABLE II

EFFECT OF DIFFERENT SUGARS ON PENICILLIN ASSAY WITH THREE STRAINS OF STAPHYLOCOCCI

Assay medium	Highest inhibiting dilution		
	S. aureus (292)	*S. albus* (457)	*S. aureus* (458)
Nutrient broth	<10	<10	10
1% glucose broth	>3200	1600	3200
1% sucrose broth	20	320	80
1% lactose broth	<10	<10	10
1% mannose broth	<10	<10	

The addition of the culture fluid to the assay tubes containing sugar increases the acidity, particularly with glucose, sucrose and mannose, an effect which is not immediate but which becomes apparent upon incubation. However, this effect is diluted out in preparing the higher tubes of the assay series, indicating that the increased bacteriostatic effect in the presence of glucose is not to be ascribed to acidity. This belief is further supported by the fact that assays made with plain nutrient broth with the pH adjusted to 6.0 or 5.2 give values similar to those at pH 6.8. Furthermore, pH does not appear to explain differences noted with different sugars.

ANTIBACTERIAL ACTION ON GROWING CULTURES

The antibacterial effect of penicillin with and without sugar was further studied in a series of quantitative determinations of organisms surviving following treatment of 16-hour cultures of staphylococci growing in plain nutrient broth. Counts of viable organisms were made by plating appropriate dilutions of the 16-hour culture, after which it was subdivided into 50-ml. portions. Further counts were made to note the effect of the various addenda after further incubation periods of 8 and 24 hours.

TABLE III

EFFECT OF PENICILLIN (CRUDE CULTURE FLUID) AND SUGARS ON GROWING CULTURES OF STAPHYLOCOCCI

Cult. No. (Test org.)	Additions to 16-hour culture of staphylococcus	Count per ml. after addition		
		At start	8 hours	24 hours
292	Penicillin (1-50 crude cult.)	345,000,000	440,000,000	760,000,000
	Penicillin + glucose	345,000,000	325,000,000	27,000,000
	Penicillin + sucrose	345,000,000	280,000,000	17,000,000
	Glucose	345,000,000	380,000,000	260,000,000
	Sucrose	345,000,000	490,000,000	360,000,000
	Control	345,000,000	560,000,000	640,000,000
457	Penicillin (1-50 crude cult.)	410,000,000	550,000,000	580,000,000
	Penicillin + glucose	410,000,000	13,000,000	<1,000,000*
	Penicillin + sucrose	410,000,000	450,000,000	150,000,000
	Glucose	410,000,000	560,000,000	340,000,000
	Sucrose	410,000,000	690,000,000	530,000,000
	Control	410,000,000	770,000,000	690,000,000

*1/1,000,000 plates practically sterile.

TABLE IV

EFFECT OF PENICILLIN (POWDER PREPARATION) AND SUGARS ON GROWING CULTURES OF STAPHYLOCOCCI

Cult. No. (Test org.)	Additions to 16-hour culture of staphylococcus	Count per ml. after addition		
		At start	8 hours	24 hours
459	Penicillin I (1-440,000)	260,000,000	257,000,000	36,600,000
	Penicillin I + glucose	260,000,000	219,000,000	8,800,000
	Penicillin II (1-2,200,000)	260,000,000	330,000,000	182,000,000
	Penicillin II + glucose	260,000,000	300,000,000	14,200,000
	Glucose	260,000,000	480,000,000	153,000,000
	Control	260,000,000	375,000,000	605,000,000
457	Penicillin I (1-440,000)	450,000,000	230,000,000	52,000,000
	Penicillin I + glucose	450,000,000	214,000,000	47,000,000
	Penicillin II (1-2,200,000)	450,000,000	455,000,000	130,000,000
	Penicillin II + glucose	450,000,000	410,000,000	67,000,000
	Penicillin I + sucrose	450,000,000	260,000,000	105,000,000
	Glucose	450,000,000	650,000,000	230,000,000
	Sucrose	450,000,000	630,000,000	650,000,000
	Control	450,000,000	795,000,000	785,000,000
458	Penicillin I (1-440,000)	170,000,000	20,000,000	*
	Penicillin I + glucose	170,000,000	*	*
	Penicillin II (1-2,200,000)	170,000,000	54,000,000	*
	Penicillin II + glucose	170,000,000	*	*
	Penicillin I + sucrose	170,000,000	*	*
	Glucose	170,000,000	780,000,000	305,000,000
	Sucrose	170,000,000	710,000,000	670,000,000
	Control	170,000,000	635,000,000	670,000,000

*1/100,000 dil. plates practically sterile.

In table III are shown results from tests with two strains of staphylococci in which crude culture fluids of *P. notatum* were used. Though differences between the test organisms are apparent, the antibacterial action of penicillin in the presence of the sugars is quite marked, indicative of a bactericidal effect, most pronounced in the case of glucose with culture 457, a pathogenic strain of *Staphylococcus albus*. Table IV gives results from tests similarly carried out on three strains of staphylococci. In these series a powdered preparation of penicillin was used at different concentrations. Here again, in spite of considerable variation in the susceptibility of various strains, the antibacterial effect of penicillin is increased in the presence of the sugars under the test conditions.

DISCUSSION

In discussing the titration method for measuring penicillin potency, Fleming (3) lays stress on the importance of an assay method giving constant and comparable results, pointing out that there is no merit in using a test which gives an enhanced value to penicillin. Our findings indicate that the addition of glucose and sucrose, particularly the former, may give very much higher values than those from plain nutrient broth, and emphasize the desirability of uniformity in testing, particularly as regards media and strain of test organism. At the same time they indicate an enhanced antibacterial effect in the presence of glucose under certain test conditions.

The precise mode of action of the sugar remains a matter for further study. The increased antibacterial effect may be presumed to be the result of a reaction of glucose with the antibacterial substance, or with other constituents of the medium, or with both. Various workers have shown that peptone is inhibitory to the bacteriostatic action of sulfonamides (2, 4, 5, 6) and of other antibacterial agents (7). In our experiments the glucose effect was exerted in the presence of peptone. There is recent evidence that inhibition of certain bacteriostatic agents may be reduced or destroyed in the presence of additional factors in the medium. Thus Oxford (7) reports that the addition of 2 per cent glucose to heart broth containing peptone increases the bacteriostatic action of penicillic acid (a mould by-product, though not related to penicillin) against *Escherichia coli*, presumably by diminishing the inhibitory effect of peptone. Of similar interest are the findings of Boroff and Bullowa (1), who report that the activity of a substance in serum capable of inhibiting sulfapyridine action on pneumococci may be destroyed by action of a soil bacillus. Additional tests are required to determine whether the glucose effect on penicillin potency is related to inhibition factors.

SUMMARY

In tests of penicillin potency of culture fluids of *Penicillium notatum* much higher values were obtained with 1 per cent glucose broth than with plain broth used for growth of the test organisms (staphylococci). A less pronounced effect was obtained with sucrose, though lactose or mannose, used at the same concen-

tration, were without effect. When added to actively growing broth cultures of staphylococci, glucose markedly increased the antibacterial action of penicillin.

ACKNOWLEDGMENTS

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A Case of Obscure Pulmonary Infection*

Observations on Lung Infection Roentgenologically Tuberculous but Bacteriologically Yielding a Pathogenic Yeast and Non-Pathogenic Acid-fast Bacillus

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Captain, R.C.A.M.C.

A CASE report has been prepared to illustrate the confusion which may arise from the presence of acid-fast saprophytes in the sputum of a case whose X-ray examination indicated pulmonary tuberculosis. The picture was further complicated by the presence of yeast in the sputum, and this latter was finally considered to be the infecting organism.

The patient, W.J., 28 years old, married, and employed as a chauffeur, had no symptoms, felt well, and was gaining weight. On applying for military service he was rejected because of the X-ray picture indicating widespread pulmonary tuberculosis.

History: Pleurisy thirteen years previously. Rheumatic fever nineteen years ago. Severe cold three months ago, with sputum which cleared in three weeks.

Family History: Mother died of tuberculosis twenty-five years previously.

Physical Examination: No symptoms or signs relative to any system were elicited. Temperature and pulse were not elevated at any time. Sputum was obtained with difficulty and in very small amounts. Tuberculin tests with human tuberculin 1:400 were negative; with 1:10, slightly positive. Bovine tuberculin produced no reaction. With avian tuberculin 1:400 he gave a definitely positive reaction.

The sedimentation rate was 3 in one hour, and 6.5 in two hours.

X-ray Findings: The lungs had a diffuse, soft mottling and some stranding in the upper two-thirds on both sides, with slightly increased density at hiluses.

Laboratory Examination

The essential features of the laboratory data are:

1. Thirty sputum-concentration examinations were made, with only one positive for acid-fast bacilli.

2. Nine gastric lavage cultures yielded acid-fast bacilli in moist orange colonies on four occasions, but no tubercle bacilli.

3. Sputum cultured on Petragnani's medium twice, grew acid-fast bacilli in moist brilliant orange-coloured colonies but no typical colonies of tubercle bacilli.

4. Sputum cultured on Sabouraud's medium, grew moist creamy colonies of *Monilia albicans*. Budding yeast cells, but no mycelia, were seen on direct smears of the sputum.

*Presented at the tenth annual Christmas meeting of the Laboratory Section, Canadian Public Health Association, held in Toronto December 17 and 18, 1941.

5. Bronchoscopic aspiration was made (fourteen months after admission). No acid-fast bacilli were obtained on smear or culture. *Monilia albicans* was cultured from the left bronchus.

6. A twenty-four hour specimen of urine was negative for acid-fast bacilli.

7. The patient gave a negative skin test with a killed suspension of *Monilia albicans* obtained from him. Agglutination tests were negative.

The yeast grew readily in 2-3 days as a profuse, moist creamy growth on Sabouraud's medium and also on blood agar. Mycelia were difficult to find on artificial medium and were first demonstrated in kidney lesions in the rabbit. They were identified as *Monilia albicans* by Dr. Rhoda W. Benham. The monilia consistently produced fatal disease in rabbits, mice and rats when given intravenously and intraperitoneally. Miliary necrotic foci laden with round and oval, doubly-contoured bodies and pus cells were found in all organs—the cortex of the kidneys, the heart and the brain showing the heaviest seeding. Intraperitoneal injection produced a granulomatous omentitis resembling tuberculosis and the lesions contained yeast cells.

The acid-fast organism appeared in about two weeks, on Petragnani's plain and glycerinated medium, as fine, rounded, moist, sticky, brilliant orange colonies, later becoming diffuse and confluent. Subcultures appeared in about four days and rapidly covered the surface of the medium.

The acid-fast bacilli failed to cause any definite parenchymal lesions in hens or rabbits injected intravenously, or in guinea pigs injected subcutaneously, either in small or large doses. The spleen harboured the organisms which were recovered on smear and culture. Local lesions were produced occasionally in guinea pigs with discharge of acid-fast bacilli and subsequent healing. The inoculated animals frequently gave a positive intradermal test with avian tuberculin 1:1 as early as four weeks, but the results were inconsistent. No reaction was produced with homologous antigen.

A study of chromogenic acid-fast bacilli from various sources, gastric washings, sputum and urine was done concurrently. Over a period of twenty-two months the incidence of this growth in routine culture of gastric washings for tubercle bacilli was 4.2 per cent (46 of 1096). Inoculation of the bacilli from these sources failed to produce disease in hens or guinea pigs, although they frequently developed skin sensitivity to avian tuberculin. From their characteristics and non-pathogenicity they were considered saprophytes.

PROGRESS

In spite of the indefinite signs, the case in the early stage was considered bilateral tuberculosis and right artificial pneumothorax was instituted and maintained for eight months. Radiologically, the lungs showed no significant change, and no symptoms developed. When the *Monilia albicans* isolated from the sputum proved pathogenic to animals, potassium iodide therapy was considered but was rejected because of the possible dangers from its use in the presence

of tuberculosis. Therefore, the patient continued on bed rest and pneumothorax treatment.

That the yeast and the acid-fast bacilli originated from the lungs was assumed but not proven. Sputum containing both organisms was obtained after having the patient rinse his mouth with a mild antiseptic wash. An attempt was made to aspirate secretion from the lungs by means of a bronchoscope, but no secretion was present and no cultures could be made. After considerable persuasion, the patient submitted to another bronchoscopic aspiration, six months later (fourteen months after admission) but the specimens obtained yielded no chromogenic bacilli, and yeast from the left bronchus only, which grew very slowly (five weeks) on Sabouraud's medium and was non-pathogenic to rabbits. Loss of pathogenicity may have been due to its chronicity in the host or to the time elapsing for its appearance on artificial medium. He was discharged fourteen months after admission with a radiological lung picture the same as on admission, having developed no symptoms beyond a trace of sputum, and having gained 12 pounds.

Discussion

The etiology of the lung lesions in this case is obscure. Infection by human tubercle bacillus is ruled out by the stability of the process over fourteen months' observation, the very weak allergic reactions to human tuberculin and the persistently negative sputum with one exception. Boeck's sarcoid was considered but as he had no involvement of skin, lymph nodes, bones, uveoparotid tract, nor apparently any tissue outside the lungs, the diagnosis was unlikely. Two distinct organisms were found in the lung secretions. The final impression, though inconclusive, is that of pulmonary moniliasis with the chromogenic acid-fast bacillus a non-pathogenic contaminant. In support of the diagnosis of pulmonary moniliasis are the low-grade infection without clinical symptoms or progression, the repeated presence of *Monilia albicans* in sputum and once in bronchial aspiration, the pathogenicity of the yeast to laboratory animals, and the absence of any other pathogenic organism. These criteria are based on those outlined by Castellani (1), by various other authors mentioned by Koerth et al (2), by the Flinns (3), and by Ikeda (4). The lack of allergic response to the skin test with killed monilia suspension is not unduly disturbing since the test with heat-killed cells is notably inconsistent due to the fact that the reacting substance appears to be thermo-labile (5).

The relationship between human, bovine and avian tubercle bacilli and various strains of saprophytic acid-fast bacilli from animal and human sources is indicated by the allergy produced to heterologous tuberculins as repeatedly demonstrated by Crawford (6), Pinner (7), and Karlson and Feldman (8). A positive avian tuberculin test in our patient led us to test several other persons including patients and nursing staff who were known to be positive to human tuberculin. Eleven of sixteen tested were positive also to avian tuberculin, several in dilutions up to 1:400. This finding tended to lessen the importance

of the patient's sensitivity to avian tuberculin. His allergy may well be due to an heterologous antigen, i.e. the chromogenic acid-fast bacilli in the lungs.

SUMMARY

A case of symptomless pulmonary disease discovered by X-ray in a young army recruit is presented. After much study the case was considered to be pulmonary moniliasis.

Sputum examination yielded (1) a non-pathogenic acid-fast bacillus, (2) *Monilia albicans* which was highly pathogenic for rabbits and mice and less so for rats.

The finding of acid-fast bacilli on sputum examination coupled with the suggestive X-ray picture led to an early erroneous diagnosis of pulmonary tuberculosis.

The case illustrates the confusion which may arise from the presence of acid-fast saprophytes in sputum and gastric washings.

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ALBERT GRANT FLEMING, M.C., M.D., D.P.H., F.R.C.S.

Photo by Jacoby

IT was with a profound sense of loss that the members of the Canadian Public Health Association learned of the passing of Dr. Albert Grant Fleming, who died in Montreal on April 9th after an illness of a few months. He had assumed the presidency only in January, at a time when measures for social security, including national health insurance, of which he was a pioneer advocate, were being brought forward for consideration by Parliament. No other public health leader was so well qualified to guide the Association in its consideration of national health problems. Since 1928, when the Association reaffirmed its objectives and assumed new responsibilities as the professional society of public health workers, the Association's committees and the Executive Council have benefited from his counsel. Because of these contributions in leadership, the Association appreciates more keenly the loss to public health in Canada occasioned by his passing.

Dr. Fleming was born in 1887 in Toronto, where he received his education. He graduated from the Faculty of Medicine, University of Toronto, in 1907 with the degree of M.B. His early interests lay in the laboratory field, and his experience in the Department of Public Health laboratories in the city of Toronto, of which he was appointed bacteriologist in 1908, formed the foundation for his broad interests in public health. He held this appointment until 1913, when he enrolled in the course leading to the Diploma in Public Health at the School of Hygiene, University of Toronto. At the completion of this work he enlisted for service with the Canadian Army Medical Corps in France. In 1918 he was awarded the Military Cross. He also served as assistant to the consultant in hygiene and sanitation to the Canadian Forces in England.

On returning to Canada in 1919 he was appointed director of laboratories for the Department of Public Health, Toronto. A year later he assumed the direction of the newly created venereal-disease division of the Department of Pensions and National Health, Ottawa. In 1921 he returned to Toronto as Deputy Medical Officer of Health, a position which he resigned in 1924 in order to direct the work of the Montreal Anti-Tuberculosis and General Health League. As a result of the interest created in public health in Montreal, re-organization of the Department was effected and Dr. Fleming was named a member of the Board of Health. In 1928 he became Professor of Public Health and Preventive Medicine at McGill University, and director of the Department. His administrative ability was recognized by the University in 1936 in his appointment as Dean of the Faculty of Medicine. He held this office until 1940, when he became Medical Director of the Bell Telephone Company of Canada, continuing to serve also as Professor of Public Health and Preventive Medicine in the University. This appointment was additional recognition of his ability and training as a leader in industrial health. A few months before his death he had been chosen to serve as a consultant in preventive medicine to the Director-General of Medical Services (Army) in Ottawa.

To national voluntary agencies—the Canadian Tuberculosis Association, the Health League of Canada, the Victorian Order of Nurses, and the Canadian National Committee for Mental Hygiene, he gave most generously of his time. From 1928 to 1936 he was Associate Secretary of the Canadian Medical Association. He served both the American and the Canadian Public Health Associations as vice-president and as a member of their governing councils. He was a member of the advisory boards of many organizations, including the Montreal Council of Social Agencies, the Commonwealth Fund of New York, the Kellogg Foundation of Battle Creek, the Mental Hygiene Institute of Montreal, the Child Welfare Association of Montreal, the Royal Edward Institute, Montreal, the Laurentian Sanatorium Association, etc. He was the author of many scientific articles and prepared numerous publications for popular health instruction.

In the thirty-five years of his professional life, Dr. Fleming achieved international recognition as a leader in public health and an authority in medical education. Of him it may well be said that he was an expert in almost every field of public health. In all of them he gave leadership and rendered outstanding service. He advanced greatly the cause of public health in Canada and enriched the lives of the many physicians who were privileged to be his students.

A CO-ORDINATED PROGRAM FOR VENEREAL-DISEASE CONTROL IN CANADA

DURING the last war the attention of public health leaders was directed to the problem of venereal diseases, particularly their incidence in the armed forces. It was recognized that infections in the forces had their origin in the civilian population. Dr. Gordon Bates led the efforts to arouse public opinion and to obtain support from the Federal Government to achieve a Canada-wide program for the control of these diseases. Under the direction of Dr. Bates

the National Council for Combating Venereal Diseases (now the Health League of Canada) did pioneer work. The menace of venereal disease served to focus attention on the need for federal action in the field of public health, and this resulted in the establishment in 1919 of the Department of Pensions and National Health—the federal department of health of Canada. In 1920 a special division for the control of venereal diseases was created in the department and a grant of $200,000 was made available to assist the provinces in establishing venereal-disease clinics. The valuable service of the voluntary health agency working in this field was recognized and financial support was given to further its activities. Thus the federal and provincial authorities undertook a co-ordinated program for the control of venereal diseases.

Public health officers are familiar with the success achieved during the years in which these arrangements obtained. Disintegration of the program followed, however, when the government grants-in-aid were discontinued in 1932 and the division of venereal-disease control in the federal department was disbanded. Efforts for the control of venereal disease continued to be made by the Department of Pensions and National Health; and as a result of representations by the Dominion Council of Health, the Health League of Canada, and other bodies, provision for the supplying of arsenicals required in the clinics throughout Canada was undertaken by the federal department in 1940.

The present war has again brought to light the urgency of an adequate program for the control of venereal diseases in Canada. The success of such a program depends on the united efforts of the medical profession, the official health authorities, police departments, and the churches. There must be provision for efficient diagnosis and treatment, the finding of contacts and their treatment when infected, and an effective program of education. There must be effective legislation for dealing with recalcitrant and other problem cases, as well as for the full exercising of police authority to stamp out organized vice and to reduce clandestine prostitution through closer supervision of beverage rooms, dance halls, etc. The church must speak of the moral issues that too often have been put aside in considerations of the problem of venereal-disease control. It is evident that success can be attained only by a Canada-wide program supported by the federal government. The great advance in the control of these diseases was made at a period when the policy of grants-in-aid was in force. It is indeed encouraging news that the grants-in-aid are being re-established and that a forward movement is to be initiated.

Outstanding leadership in the effort is assured in the appointment of Lieutenant-Colonel Donald H. Williams as director of venereal-disease control measures in the armed services. He will also serve as director of the venereal-disease control division of the Department of Pensions and National Health. Not only is the co-ordination of efforts by the three branches of the armed services significant, but the program is unique in providing a unified control of the entire population, civilian and military. It will be recalled that Colonel Williams, as director of the work conducted by the Provincial Board of Health, achieved great success in British Columbia in his fearless efforts to control prostitution and to reduce the incidence of these diseases.

BOOKS AND REPORTS

The 1942 Year Book of General Medicine. *Edited by George F. Dick, M.D., and others. Chicago: The Year Book Publishers Inc., 1942. 848 pages. $3.00 (U.S. funds).*

WHEN it is remembered that textbooks become obsolete in some respects soon after they are printed, the value of an annual presentation of current developments is obvious. The Year Book of General Medicine serves as a useful and comprehensive reference for a wide field of medicine. The survey of medical literature contained in the 1942 edition is, as always, a highly satisfactory one. Every practitioner, general or specialist, is certain to find in these pages information of interest and value to him. Because of the great diversity of subject-matter abstracted, an adequate review is almost impossible.

In this edition one or two inaccuracies have been detected. For instance, it is stated that alum-precipitated toxoid contains a greater proportion of foreign protein than does plain toxoid, which is not necessarily true. In another section the iso-agglutinins involved in blood-grouping are referred to as A and B, which is contrary to accepted practice. The capital letters should be reserved for the iso-agglutinogens and the small letters, a and b, for the iso-agglutinins. Again, it is stated that Group AB may be mistaken for Group O blood if weak test sera are used. This could happen, but the more common error would be to mistake Group AB for Group B.

F. O. Wishart

Introduction to Parasitology. *By A. S. Pearse. Springfield, Ill.: Charles C Thomas, 1942. 357 pages. $3.75 (U.S. funds).*

THIS book contains short accounts of many animals having parasitic habits, included among which are protozoan parasites of plants and vertebrates, such as cuckoos and vampire bats. Herein lies a weakness of the book, for in covering such a wide field in relatively small space, the parasites can be described and discussed in general terms only. Consequently, it is almost impossible to make general statements about certain groups which hold good for all members. Moreover, some of the terminology is rather specialized for an introductory text book and is likely to confuse the beginner. The parasites of man, with the possible exception of those responsible for malaria and hookworm, are not considered in sufficient detail to make the book useful to the medical student. The zoologist too is likely to be disappointed that the subject has been treated so generally. The book contains over four hundred line drawings, and bibliographies are given at the end of each chapter.

A. Murray Fallis

Mental Hygiene for Community Nursing. *By Eric Kent Clarke. Minneapolis: The University of Minnesota Press, 1942. 262 pages. $3.50 (U S. funds).*

UNDER the title, *A Challenge to the Public Health Nurse*, the introductory chapter of this book affirms the author's broad concept of public health nursing, as well as his sympathetic understanding of the public health nurse in her endeavor to meet the problems with which she is confronted daily. Added to these essentials is a rare skill in elucidation: complex circumstances are made clear by means of a direct approach and the use of simple terms.

In the education of the nurse, there is a plea for greater emphasis on human relationships, the care of the mentally ill, and the mental hygiene aspects of every situation.

In the eleven chapters of the book, the author discusses the needs of and difficulties of the pre-school child, the child at school, the handicapped child, the adolescent, the family, the

convalescent and chronic invalid, the psychoneurotic, the psychotic, community care of the feeble-minded, and the mental hygiene of the community nurse. Brief case histories illustrate the subjects under discussion. Seven pages are devoted to suggested reading, and there is a detailed index.

This book is a guide that public health nurses will want to own. It doubtless will prove to be a much-used reference. *Edna L. Moore*

CURRENT HEALTH LITERATURE

A Milk-borne Outbreak of Diphtheria

In April, 1941, diphtheria was diagnosed in 2 children in a farmer's family in East Yorkshire and confirmed by isolation of *C. diphtheriae gravis* from their throat swabs. Another child had been ill with a sore throat a week prior to this but had not been seen by a doctor. Nasal and throat swabs were taken from the remainder of the family, father, mother and fourth child. *C. diphtheriae gravis* was isolated from the throat swab of the mother. She and the children were released from the isolation hospital at the beginning of June after 4 consecutive nose and throat cultures had been reported negative.

Milk from this farm was sold privately to 3 houses and to a distributor in a nearby town. The farmer did the milking himself and his wife strained the milk and washed the utensils. Between July 10th and 16th, 7 cases of diphtheria occurred in the town, 4 of them in the houses supplied direct by the farmer and 3 in families supplied by the distributor. A sample of the raw milk was cultivated and *C. diphtheriae gravis* isolated. Two further samples of milk were examined later, one not handled by the farmer's wife and the second strained in the usual way by her. This second sample was positive and the first negative. The fourth child subsequently developed clinical diphtheria. The farmer's wife gave a history of having had a sore throat in March, 1941.

The use of a medium containing tellurite facilitated the isolation of the diphtheria bacillus in this outbreak, positive cultures being obtained when Loeffler's medium gave negative results.

William Goldie and E. C. G. Maddock, Lancet, 1943, Feb. 27, p. 285.

A Milk-borne Epidemic of Brucellosis

The outbreak of brucellosis reported in this paper is probably the largest on record in which milk served as the vehicle of infection, 77 cases being detected. The responsible organism, *Brucella suis*, was isolated in 13 of 29 attempts. Attention was directed to the milk supply by the occurrence of multiple cases and especially of 4 in one family. In all, 51 families gave evidence of infection either by clinical illness or agglutination tests, and multiple cases occurred in 15 homes.

On one dairy farm 4 animals were found which gave positive reactions and from the milk of 2 of these *Brucella suis* was isolated. Agglutination tests on 24 sows on this farm were positive in 11 instances. Hogs and dairy cows had been allowed to mingle freely on this farm. No further milk from this source was distributed and pasteurization was introduced. In spite of this, positive agglutination reactions continued to occur, indicating a prolonged incubation period.

I. H. Borts, D. M. Harris, M. F. Joynt, J. R. Jennings and Carl F. Jordan, J.A.M.A., 1943, *121*: 319.

An Outbreak of Streptococcic Sore Throat in an Army Camp

Some 3,000 men were stationed at the army camp involved in this outbreak of streptococcic sore throat. In the first two days commencing June 17, 1942, 291 cases occurred, with a

total of 341 cases by June 21st. The explosiveness of the epidemic seemed to indicate massive ingestion of streptococci, most likely from some article of food, though proof of this was not obtained. Milk seemed to be ruled out as the vehicle of infection.

Clinically the cases were typical of streptococcal throat infection but few of the cases were dangerously ill and there were no deaths. Approximately one-fourth of the cases had a classic scarlatinal rash with desquamation, some had a transient exanthem, and in the majority no rash was seen. Septic complications were practically absent. Mild arthritic symptoms were present in a small percentage of cases but could not be interpreted as rheumatic fever. Renal complications were not in evidence. Convalescence to complete physical fitness was slow.

Cases were sampled at random for throat cultures and group A haemolytic streptococcus, Griffith subtype 15, was isolated in each instance. Clinical severity of the disease did not seem to be related to the presence or absence of rash. The incidence of the condition was lower among those who had had a tonsillectomy, being only 16 per cent among 127 examined. Cultures were taken from 36 convalescents three weeks after the onset and 53 per cent were positive for haemolytic streptococci which were the same type as originally isolated, except in one instance. In spite of returning large numbers of convalescent carriers into the uninfected group, only one further case developed. Sulfonamide therapy was used but its efficacy could not be definitely ascertained.

Arthur L. Bloomfield and Lowell A. Rantz, J.A.M.A., 1943, *121*: 315.

Epidemic Keratoconjunctivitis

THE clinical, epidemiologic and experimental data presented in this paper are based on a study of 80 cases. Diagnostic features are: hyperaemia and swelling of the palpebral conjunctiva; congested and hyperaemic bulbar conjunctiva; oedema of the lids and occasionally the appearance of pseudomembranes, usually on the conjunctiva of the lower lid. Of great help in the diagnosis are the absence of conjunctival discharge and the presence of enlarged preauricular glands. The second eye became involved in less than half the cases and usually less severely. Corneal infiltrations occurred in 42 cases, with some impairment of vision in 14 cases. Absorption of corneal opacities had not occurred in a period of 6 months. Systemic manifestations consisted of headache in 49 patients and malaise in 26.

Disability varied from 1 to 8 weeks for 57 of the patients, the greatest time loss being among those with severe corneal involvement.

The first case was seen on January 27, 1942, and the last on September 13, with a peak occurring in April and May and again in August. Occupation did not seem to be a factor except that in 70 cases the condition developed following ocular trauma or inflammation. However, only 10 per cent of patients treated for ocular trauma or inflammation during the period of the epidemic developed keratoconjunctivitis. The mode of spread of the disease was not discernible. It is emphasized that physicians should observe meticulous hygienic precautions as to their hands and instruments in order to avoid spreading the infection to susceptible persons.

Experimental.—In 2 of 9 attempts a filtrable virus was isolated which appeared to be identical with that isolated previously. Convalescent sera from 19 patients tested were found to possess appreciable neutralizing activity for a virus isolated in another outbreak. In 6 of the 19 cases serum was obtained early during the course of the disease and later during convalescence. In each instance the second serum sample showed a marked increase in neutralizing capacity.

Murray Sanders, F. D. Gulliver, L. L. Forchheimer and R. C. Alexander, J.A.M.A., 1943, *121*: 250.